Connecting via Winsock to STN

Welcome to STN International! Enter x:x

LOGINID:SSSPTA1644PNH

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

* * * * * * * * * * Welcome to STN International * * * * * * * * * *

| | | | |
|---|---|---|---|
| NEWS | 1 | | Web Page URLs for STN Seminar Schedule - N. America |
| NEWS | 2 | | "Ask CAS" for self-help around the clock |
| NEWS | 3 | JAN 27 | Source of Registration (SR) information in REGISTRY updated and searchable |
| NEWS | 4 | JAN 27 | A new search aid, the Company Name Thesaurus, available in CA/CAplus |
| NEWS | 5 | FEB 05 | German (DE) application and patent publication number format changes |
| NEWS | 6 | MAR 03 | MEDLINE and LMEDLINE reloaded |
| NEWS | 7 | MAR 03 | MEDLINE file segment of TOXCENTER reloaded |
| NEWS | 8 | MAR 03 | FRANCEPAT now available on STN |
| NEWS | 9 | MAR 29 | Pharmaceutical Substances (PS) now available on STN |
| NEWS | 10 | MAR 29 | WPIFV now available on STN |
| NEWS | 11 | MAR 29 | New monthly current-awareness alert (SDI) frequency in RAPRA |
| NEWS | 12 | APR 26 | PROMT: New display field available |
| NEWS | 13 | APR 26 | IFIPAT/IFIUDB/IFICDB: New super search and display field available |
| NEWS | 14 | APR 26 | LITALERT now available on STN |
| NEWS | 15 | APR 27 | NLDB: New search and display fields available |
| NEWS | 16 | May 10 | PROUSDDR now available on STN |
| NEWS | 17 | May 19 | PROUSDDR: One FREE connect hour, per account, in both May and June 2004 |
| NEWS | 18 | May 12 | EXTEND option available in structure searching |
| NEWS | 19 | May 12 | Polymer links for the POLYLINK command completed in REGISTRY |
| NEWS | 20 | May 17 | FRFULL now available on STN |

| | |
|---|---|
| NEWS EXPRESS | MARCH 31 CURRENT WINDOWS VERSION IS V7.00A, CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP), AND CURRENT DISCOVER FILE IS DATED 26 APRIL 2004 |
| NEWS HOURS | STN Operating Hours Plus Help Desk Availability |
| NEWS INTER | General Internet Information |
| NEWS LOGIN | Welcome Banner and News Items |
| NEWS PHONE | Direct Dial and Telecommunication Network Access to STN |
| NEWS WWW | CAS World Wide Web Site (general information) |

Enter NEWS followed by the item number or name to see news on that specific topic.

All use of STN is subject to the provisions of the STN Customer agreement. Please note that this agreement limits use to scientific research. Use for software development or design or implementation of commercial gateways or other similar uses is prohibited and may result in loss of user privileges and other penalties.

* * * * * * * * * * * * * STN Columbus * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 08:41:54 ON 25 MAY 2004

=> file medline embase biosis scisearch caplus

| COST IN U.S. DOLLARS | SINCE FILE ENTRY | TOTAL SESSION |
|---|---|---|
| FULL ESTIMATED COST | 0.21 | 0.21 |

FILE 'MEDLINE' ENTERED AT 08:42:04 ON 25 MAY 2004

FILE 'EMBASE' ENTERED AT 08:42:04 ON 25 MAY 2004


FILE 'BIOSIS' ENTERED AT 08:42:04 ON 25 MAY 2004


FILE 'SCISEARCH' ENTERED AT 08:42:04 ON 25 MAY 2004


FILE 'CAPLUS' ENTERED AT 08:42:04 ON 25 MAY 2004


```
=> s treatment
L1     7839775 TREATMENT

=> s l1 and allergy
L2       39758 L1 AND ALLERGY

=> s l2 and modified allergen
L3          86 L2 AND MODIFIED ALLERGEN

=> dup remove l3
PROCESSING COMPLETED FOR L3
L4           48 DUP REMOVE L3 (38 DUPLICATES REMOVED)

=> s l4 and IgE epitope
L5            0 L4 AND IGE EPITOPE

=> s l4 and point mutation
L6            0 L4 AND POINT MUTATION

=> d l4 1-48 cbib abs

L4   ANSWER 1 OF 48  CAPLUS  COPYRIGHT 2004 ACS on STN
2004:107701  Document No. 140:269092  Strategies for converting allergens into
     hypoallergenic vaccine candidates.  Vrtala, Susanne; Focke-Tejkl,
     Margarete; Swoboda, Ines; Kraft, Dietrich; Valenta, Rudolf (Department of
     Pathophysiology, University of Vienna, Vienna, A-1090, Austria).  Methods
     (San Diego, CA, United States), 32(3), 313-320 (English) 2004.  CODEN:
     MTHDE9.  ISSN: 1046-2023.  Publisher: Elsevier Science.
AB   A review.  Specific immunotherapy is based on the administration of
     increasing doses of allergens to allergic patients with the aim of
     inducing a state of antigen-specific unresponsiveness.  Specific
     immunotherapy is one of the few causative treatment approaches
     for Type I allergy but may cause numerous side effects,
     including local inflammatory reactions, systemic manifestations (e.g.,
     asthma attacks) and in the worst case, anaphylactic shock which may lead
     to death.  Several attempts have been made in the past to reduce the rate
     of side effects.  They included the chemical modification of allergen exts.
     to reduce their allergenic activity and the adsorption of allergen exts.
     to adjuvants to prevent the systemic release of allergens after
     administration.  During the last decade, cDNAs coding for the most
     relevant allergens have been isolated and the corresponding allergens have
     been produced as recombinant mols.  Using allergen-encoding cDNAs, the
     amino acid sequence of allergens or purified recombinant allergens several
     strategies can now be applied to produce allergen derivs. with reduced
     allergenic activity for allergy vaccination in a controlled and
```

reproducible manner. Currently, allergen-encoding cDNAs are used to engineer recombinant hypoallergenic allergen derivs. According to the amino acid sequences and exptl. epitope mapping data, synthetic peptides representing T- or B-cell epitopes are produced and purified recombinant allergens are coupled to novel adjuvants for vaccine formulation. In this article, strategies for the production and evaluation of allergen derivs. with reduced allergenic activity for **allergy** vaccination are described. These new vaccines hold great promise to improve the current practice of allergen-specific immunotherapy and maybe also used for prophylactic vaccination in the future.

L4 ANSWER 2 OF 48 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 1
2004:192874 The Genuine Article (R) Number: 774KU. New strategies in the **treatment** and prevention of allergic diseases. Haitchi H M (Reprint); Holgate S T. Univ Southampton, Sch Med, Div Infect Inflammat & Repair Resp Cell & Mol Bio, Southampton Gen Hosp, Level D, Ctr Block, Mail Point 810, Southampton SO16 6YD, Hants, England (Reprint); Univ Southampton, Sch Med, Div Infect Inflammat & Repair Resp Cell & Mol Bio, Southampton Gen Hosp, Southampton SO16 6YD, Hants, England. EXPERT OPINION ON INVESTIGATIONAL DRUGS (FEB 2004) Vol. 13, No. 2, pp. 107-124. Publisher: ASHLEY PUBLICATIONS LTD. UNITEC HOUSE, 3RD FL, 2 ALBERT PLACE, FINCHLEY CENTRAL, LONDON N3 1QB, ENGLAND. ISSN: 1354-3784. Pub. country: England. Language: English.
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Allergic diseases (AD) are more prevalent today than 30 years ago but over the same period, few novel efficacious drugs have been discovered to treat, control or even cure these disorders. Topical or systemic glucocorticosteroids combined with symptom-relieving medications, such as beta(2)-adrenoceptor agonists, leukotriene inhibitors or antihistamines, are still the mainstay of antiallergic **treatment.** Modified glucocorticosteroids with less adverse effects, better bronchodilators and new selective mediator inhibitors may improve symptom control in the future. only specific immunotherapy has shown potential for long-lasting disease-modifying effects. Immunomodulation is a therapeutic goal, aiming to modify the dominant helper T cell Type 2 inflammation to a helper T cell Type 1 response using **modified allergens**, mycobacteria or CpG oligodeoxynucleotides. Humanised monoclonal anti-IgE antibodies are an exciting new immunomodulatory medication that are expected to reach the clinical practice and have recently been licensed in Australia and the US. Advances in molecular, cellular and genetic research of the immunopatho-physiology of AD have led to the development of new antagonists for cytokines, chemokines, receptors, second messengers and transcription factors that may become available for clinical use in the next 10 years. Specific diets supplemented with antioxidants or probiotics need further study but offer promise as safe and cheap preventative medicine. The strong genetic component of AD and the Human Genome Project have opened a new field of research, and modification or replacement of target genes has a curative potential with exciting new therapeutic developments in the years ahead.

L4 ANSWER 3 OF 48 CAPLUS COPYRIGHT 2004 ACS on STN
2003:242373 Document No. 138:270285 Recombinant allergen with reduced IgE binding but undiminished T-cell antigenicity as immunotherapeutic of type I **allergy.** Deweerd, Nicole; Singh, Mohan Bir; Bhalla, Prem L.; Swoboda, Ines (The University of Melbourne, Australia). PCT Int. Appl. WO 2003025009 A1 20030327, 66 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-AU1261 20020913. PRIORITY: AU 2001-7792 20010920.

AB The present invention relates generally to reagents useful in the immunotherapeutic or immunoprophylactic **treatment** of allergic diseases. More particularly, the present invention provides **modified allergens** exhibiting reduced IgE interactivity including reduced IgE production-stimulatory activity, while retaining T-cell antigenicity, which are useful in the immunomodulation of type I allergic disease conditions. The allergens comprise substitution, deletion or addition mutants or variants of Lol p 5, Phl p 5, Pao p 5 and immunol. related allergens. The present invention further contemplates a method of immunomodulation of allergic diseases such as type I allergic disease conditions by the administration of **modified allergens** exhibiting reduced IgE interactivity while retaining T-cell antigenicity.

L4 ANSWER 4 OF 48 MEDLINE on STN DUPLICATE 2
2003533508. PubMed ID: 14612675. Can we genetically engineer safer and more effective immunotherapy reagents?. Westritschnig Kerstin; Valenta Rudolf. Current opinion in allergy and clinical immunology, (2003 Dec) 3 (6) 495-500. Journal code: 100936359. ISSN: 1528-4050. Pub. country: United States. Language: English.

AB SUMMARY: PURPOSE OF REVIEW Progress in allergen-specific immunotherapy, the only causative form of **allergy treatment**, was limited by the lack of defined allergen molecules for vaccine formulation. Today the genetic informations for the most common allergens have been obtained. Here we review recombinant allergen-based technologies for the improvement of diagnosis and therapy of **allergy.**RECENT FINDINGS Numerous strategies, including the genetic engineering of allergens for reduction of allergenic activity, have been developed to improve allergen-specific immunotherapy. Genetically **modified allergen** derivatives with reduced allergenic activity, preserved T cell epitope repertoire and retained immunogenicity have been characterized in vitro and in vivo.SUMMARY Based on the review of the recently published data we argue that it is possible to genetically engineer safer and more effective immunotherapy reagents.

L4 ANSWER 5 OF 48 CAPLUS COPYRIGHT 2004 ACS on STN
2003:143045 Document No. 138:236376 Immunotherapy of allergic bronchopulmonary aspergillosis: A clinical and experimental approach. Svirshchevskaya, E. V.; Kurup, V. P. (Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia). Frontiers in Bioscience, 8, S92-S101 (English) 2003. CODEN: FRBIF6. ISSN: 1093-4715. URL: http://WWW.bioscience.org/2003/v8/s/996/pdf.pdf Publisher: Frontiers in Bioscience.

AB A review. Allergic bronchopulmonary aspergillosis (ABPA) is a severe allergic pulmonary complication caused by the saprophytic fungus Aspergillus fumigatus. The present review examines the pathogenesis of this disease describing in detail the role of innate and acquired immunity in the induction of sensitivity to A. fumigatus. Different approaches in developing specific immunotherapeutic **treatments** such as induction of energy, regulatory cells, a switch from Th2 to Th1 type of immune response, CpG and genetic immunization and the usage of altered peptides or **modified allergens** are critically examined

L4 ANSWER 6 OF 48 MEDLINE on STN DUPLICATE 3
2003483241. PubMed ID: 14561174. Immunomodulatory **treatment** strategies for allergic diseases. Varga Eva-Maria; Nouri-Aria Kayhan; Till Stephen J; Durham Stephen R. (Division of Respiratory and Allergic Diseases, Department of Pediatrics, University of Graz, A-8036 Graz, Auenbruggerplatz 30, Austria.. evamaria.varga@kfunigraz.ac.at) . Current drug targets. Inflammation and allergy, (2003 Mar) 2 (1) 31-46. Ref: 131. Journal code: 101160019. ISSN: 1568-010X. Pub. country: Netherlands. Language: English.

AB Over the last decades the prevalence of allergic disorders, such as hayfever and asthma has increased worldwide, mostly in westernised countries where up to 20 % of the population are affected. The "hygiene hypothesis" suggests that modernised lifestyles such as improved housing

conditions, altered dietary habits and smaller family sizes may be responsible for the decrease in infectious and the increase in allergic diseases. Childhood atopic diseases, like eczema, food **allergies** and recurrent wheezy bronchitis represent a considerable health problem and a major socioeconomic burden due to the chronicity of these disorders. In recent years, a better understanding of the immunopathogenesis of allergic diseases has evolved, which has contributed to the development of novel more targeted forms of therapy. Allergen injection immunotherapy is the only **treatment** in current use with the potential for modifying the course of allergic disease. In order to better target mucosal **allergies**, new approaches of administering allergen, via the sublingual or intranasal route, are being developed. The use of **modified allergens**, allergen peptides, DNA immunization and the use of novel adjuvants represent alternatives to conventional immunotherapy with potential for improved efficacy with less side effects. For atopic asthma, novel **treatment** strategies aim at locally targeting inflamed airways. Nebulized monoclonal blocking antibodies and soluble interleukin receptors against "Th(2)-type" cytokines have been designed. An alternative approach has been the administration of "Th(1) -type" cytokines. Although, immunomodulatory strategies provide a promising outlook for the **treatment** of allergic patients, more studies are needed in the future to address issues of efficacy, safety and long-term effects of altered immune responses.

L4 ANSWER 7 OF 48 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 4
2003:180663 Document No.: PREV200300180663. Studies on the experimental allergic rhinitis induced by Japanese cedar pollen: Role of cysteinyl leukotrienes in nasal allergic symptoms. Mizutani, Nobuaki [Reprint Author]. Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto, 607-8414, Japan. mizutani@mb.kyoto-phu.ac.jp. Yakugaku Zasshi, (January 2003) Vol. 123, No. 1, pp. 1-8. print.
ISSN: 0031-6903 (ISSN print). Language: Japanese.
AB Cysteinyl leukotrienes (CysLTs: LTC4, LTD4, and LTE4) are a family of potent inflammatory mediators that appear to contribute to the pathophysiologic features of allergic rhinitis. Because **treatment** with a CysLT1 receptor antagonist and a 5-lipoxygenase inhibitor **modified allergen**-induced nasal blockage in patients with allergic rhinitis, and CysLTs were detected in nasal cavity lavage fluid, it has been suggested that CysLTs act as significant inflammatory mediators in allergic rhinitis. The role of CysLTs was evaluated in our experimental allergic rhinitis model in sensitized guinea pigs which shows biphasic nasal blockage, sneezing and nasal hyperresponsiveness to LTD4 induced by repetitive inhalation challenge with Japanese cedar pollen. In this model, the CysLT1 receptor antagonist pranlukast suppressed the late-phase nasal blockage but not early blockage and sneezing. Nasal hyperresponsiveness (nasal blockage) to LTD4 was largely blocked by pranlukast, naphazoline, and Nomega-nitro-L-arginine-methyl ester. The results demonstrate that nasal blockage induced by CysLTs is-mainly due to dilatation of nasal blood vessels, which can be induced by the nitric oxide produced through CysLT1 receptor activation. On the other hand, when pollen inhalation challenge was performed in the presence of nasal hyperresponsiveness, antigen-induced biphasic nasal blockage and sneezing were considerably enhanced and CysLTs contributed to both symptoms, suggesting that nasal hyperresponsiveness induces aggravation of antigen-induced nasal symptoms. The results presented in this study further suggest that our model is a good representative of human allergic rhinitis and offer evidence that CysLTs are chemical mediators mainly responsible for allergic nasal symptoms.

L4 ANSWER 8 OF 48 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2003:214491 Document No.: PREV200300214491. Systemic reactions to allergen immunotherapy: A review of the literature. Nettis, Eustachio [Reprint Author]; Giordano, Daniela; Ferrannini, Antonio; Tursi, Alfredo. Department of Clinical Immunology and Allergology, Policlinico, Piazza

Giulio Cesare, 11, 70124, Bari, Italy. e.nettis@allergy.uniba.it. Immunopharmacology and Immunotoxicology, (February 2003) Vol. 25, No. 1, pp. 1-11. print.
CODEN: IITOEF. ISSN: 0892-3973. Language: English.

AB Since its introduction the safety of specific immunotherapy (SIT) was assessed by many well-designed studies. SIT is accepted as an effective **treatment** of allergic diseases despite the occurrence of side-effects, among which systemic reactions (SRs) are the most dangerous. The reported frequency of SRs after SIT varies among the studies and several factors influence it. Asthma is a particular risk factor for systemic side-effects. Furthermore, SRs occur more often in patients with high allergen sensitivity as determined by skin testing or RAST. Making dosage errors is also considered to be a high risk. It is reported that reactions are more common during rush and clustered induction **treatment**, whereas a significantly lower incidence of SRs occurred with the use of standardized **modified allergen** vaccines than with aqueous extracts. On the basis of valuable guidelines, precautions to minimize the risk of SRs from SIT were recommended. Injections should be given or supervised by doctors well-trained in this form of **treatment** in a clinic where there is the immediate availability of a resuscitative equipment. Consideration should be given to evaluate the patient's conditions and to monitor subjects for a minimum of 30 minutes after the injections. Therefore, if appropriately done, the risk of SIT is negligible.

L4 ANSWER 9 OF 48 CAPLUS COPYRIGHT 2004 ACS on STN

2002:899107 Document No. 138:121698 Clinical aspects of food **allergy**. Papageorgiou, P. S. (Athens University School of Medicine, P & A. Kyriakou Children's Hospital, Allergy Unit, Athens, 115 27, Greece). Biochemical Society Transactions, 30(6), 901-906 (English) 2002. CODEN: BCSTB5. ISSN: 0300-5127. Publisher: Portland Press Ltd..

AB A review. Food **allergy** affects 2.5% of adults and 6-8% of children, and is a leading cause of life-threatening anaphylactic episodes. Food **allergy** is defined as an adverse reaction to foods that is mediated immunol. and involves specific IgE or non-IgE mechanisms. In this review only IgE-related food **allergy** will be considered. Many food allergens are glycoproteins, but they do not share any striking biochem. similarities. The definition of many food proteins at the mol. level has tremendously facilitated our understanding of clin. syndromes and seemingly bizarre observations. Clin. manifestations of food **allergy** include symptoms of the gastrointestinal, cutaneous and respiratory systems, as well as systemic anaphylaxis. The diagnosis of food **allergy** involves a stepwise approach, including medical history taking, demonstration of specific IgE and confirmation by oral food challenge. The management of the food-allergic patient at present consists of avoidance of the culprit food and education, while future advances may include specific immunotherapy with **modified allergens** or DNA vaccination.

L4 ANSWER 10 OF 48 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

2003:227012 The Genuine Article (R) Number: 652VZ. Hymenoptera sensitivity: Diagnosis and **treatment.** Ditto A M (Reprint). Northwestern Univ, Feinberg Sch Med, Dept Med, Div Allergy Immunol, 303 E Chicago Ave, S-207, Chicago, IL 60611 USA (Reprint); Northwestern Univ, Feinberg Sch Med, Dept Med, Div Allergy Immunol, Chicago, IL 60611 USA. ALLERGY AND ASTHMA PROCEEDINGS (NOV-DEC 2002) Vol. 23, No. 6, pp. 381-384. Publisher: OCEAN SIDE PUBLICATIONS INC. 95 PITMAN ST, PROVIDENCE, RI 02906 USA. ISSN: 1088-5412. Pub. country: USA. Language: English.
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Hymenoptera anaphylaxis is responsible for 50 deaths annually. This may be an underestimation because not all anaphylactic episodes are recognized or reported. Unexplained deaths at the poolside or on golf courses, as well as those attributed to cardiac causes, may be caused by unrecognized anaphylaxis. Venom immunotherapy (VIT) is highly effective in reducing an individual's risk of anaphylaxis. This review will discuss the diagnosis

and **treatment** of hymenoptera sensitivity, patient selection for IT, standard VIT, and **modified allergens.**

L4 ANSWER 11 OF 48 MEDLINE on STN DUPLICATE 5
2003483236. PubMed ID: 14561182. Peptide-based vaccines in the **treatment** of specific **allergy.** Alexander Clare; Kay A Barry; Larche Mark. (Allergy & Clinical Immunology, Faculty of Medicine, Imperial College, Dovehouse Street, London SW3 6LY, UK. ) Current drug targets. Inflammation and allergy, (2002 Dec) 1 (4) 353-61. Ref: 77. Journal code: 101160019. ISSN: 1568-010X. Pub. country: Netherlands. Language: English.

AB The efficacy of conventional allergen-specific immunotherapy (SIT) for allergic conditions and venom hypersensitivity is well documented. However it's use is limited due allergic side effects including anaphylaxis and the difficulty of standardising proteins in complex allergenic mixtures. The aim of new therapeutic strategies is to circumvent these limitations and approaches include allergen non-specific therapy, such as anti-IgE and anti-cytokine therapy and other allergen specific techniques including the peptide based vaccines (PBV), **modified allergens** (allergoids) and DNA vaccines. PBV are small linear peptide fragments containing T cell epitopes which are designed to reduce the ability to cross link antigen-specific IgE. Studies in animal models have confirmed proof of principle demonstrating the induction of hyporesponsiveness using high doses of peptides. However, the principle limitation to clinical use of PBV is the polymorphism of HLA class II molecules. There are ongoing clinical studies using peptide-based vaccines for cat, bee and grass **allergies**--looking at both immunological mechanisms and clinical outcome measures. The mechanisms underlying the efficacy of PBV appear to be similar to those described for classical immunological tolerance. Thus, the peptides may induce anergy due to absence of co-stimulation, activation-induced cell death, a switch from a Th2 to a Th1 cytokine profile, the induction of regulatory T cells or combinations of these mechanisms. Successful immunotherapy, in bee sensitive individuals, is associated with the elaboration of IL-10. Clonal deletion is unlikely as an overall mechanism as there is evidence that the subsequent in vitro response to associated, non-injected, peptides can be suppressed. Mechanistic studies continue to provide insight into the mode of action of whole allergen and peptide-based immunotherapy. Clinical studies designed on the basis of these observations hold the promise of safer vaccines with improved efficacy. Whether this strategy can be used for **allergy** to complex allergen mixtures such as dust mites will need further evaluation.

L4 ANSWER 12 OF 48 CAPLUS COPYRIGHT 2004 ACS on STN
2001:608578 Document No. 136:84234 Hunting the magic bullet in immunotherapy: New forms of old **treatment** or something completely different?. Fearby, S.; Frew, A. J. (University Department of Medical Specialities, Southampton General Hospital, Southampton, SO16 6YD, UK). Clinical and Experimental Allergy, 31(7), 969-974 (English) 2001. CODEN: CLEAEN. ISSN: 0954-7894. Publisher: Blackwell Science Ltd..

AB A review discussing immunotherapy for allergic diseases using recombinant allergens, chemical **modified allergens** (allergoids), adjuvants, and peptides and IgE-binding haptens.

L4 ANSWER 13 OF 48 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 6
2001418237 EMBASE [Allergen immunotherapy - A position paper of the German society for allergology and clinical immunology]. DIE SPEZIFISCHE IMMUNTHERAPIE (HYPOSENSIBILISIERUNG) MIT ALLERGENEN: POSITIONSPAPIER DER DEUTSCHEN GESELLSCHAFT FUR ALLERGOLOGIE UND KLINISCHE IMMUNOLOGIE, INHALTLICH ABGESTIMMT MIT DEM ARZTEVERBAND DEUTSCHER ALLERGOLOGEN. Kleine-Tebbe J.; Fuchs Th.; Klimek L.; Kuhr J.; Lepp U.; Niggemann B.; Rakoski J.; Renz H.; Saloga J.; Simon J.. Dr. J. Kleine-Tebbe, Schlossstrasse 51, D-14059 Berlin, Germany. Allergologie 24/11 (535-544)

2001.
Refs: 37.
ISSN: 0344-5062. CODEN: ALLRDI. Pub. Country: Germany. Language: German.
Summary Language: English; German.

AB Mechanisms of allergen immunotherapy (AIT) are complex, inducing numerous immunological effects. Successfull AIT is most likely based on a functional switch of and tolerance induction in specific T cells downregulating allergic hypersensitivity and inflammation. Subcutaneous AIT for allergic rhinoconjunctivitis and allergic asthma has been successfully assessed in controlled studies with several clinically important allergens (i.e. birch-, grass- and mugwortpollen, dust mites, animal dander) and has shown convincing clinical efficacy. Considered as the only causal **treatment** besides allergen avoidance at present, AIT can alter the natural course of allergic diseases. Hymenoptera venom hypersensitivity (to bee- and wasp venom) treated with AIT gives the best results compared to AIT with other allergens. AIT is indicated in patients with IgE-mediated sensitizations and corresponding clinical symptoms to allergens, which do not or hardly permit allergen avoidance and which are availabe as suitable extracts. Decisions about indication and allergen selection should only be made by a physician with certified training or qualified knowledge and skills in allergology. AIT is administered by physicians experienced in this therapy. After addressing tolerability and present status of health, the recommended or individually adjusted dose is injected and precisely documented, followed by a mandatory waiting period of 30 minutes. Indication for and application of AIT in children are quite similar compared to the **treatment** of adults. Children tolerate AIT very well and benefit especially from its immunomodulatory effects. Risk factors for and results of unwanted systemic effects can effectively be minimized by training of the staff members involved adhering to safety standards and immediate emergency **treatment. Modified allergens**, recombinant proteins and immunomodulatory adjuvants created by basic research are promises for an improved efficacy of AIT with reduced unwanted effects in the future.

L4 ANSWER 14 OF 48 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 7

2001120857 EMBASE [Peanut **allergy**]. ALLERGIE A L'ARACHIDE. Dutau G.; Rance F.. G. Dutau, Unite des maladies respiratoires, Hopital des Enfants, 330, avenue de Grande-Bretagne, 31026 Toulouse Cedex 3, France. Revue Francaise d'Allergologie et d'Immunologie Clinique 41/2 (187-198) 2001.
Refs: 98.
ISSN: 0335-7457. CODEN: RFAIBB. Pub. Country: France. Language: French.
Summary Language: English; French.

AB Peanut **allergy**, which is frequent in the United States and was much less so in Europe up to the mid-eighties, has become a major problem in many industrialized countries. Peanut consumption is high in Eastern Europe, the United Kingdom, The Netherlands, Germany and France. The frequency of peanut **allergy** is between 0.5 and 0.7% in the general population. Two million Americans are now thought to be affected. In France peanuts are one of the most frequent allergens, lying second (27.4 %) to egg in food **allergies** in children, and holding first place in food **allergies** in children aged over 3 years. Sensitization occurs through ingestion, contact even if indirect, and inhalation. The symptoms, which affect the skin and the respiratory or gastrointestinal tract, appear a few minutes to a few hours after exposure. Serious reactions (anaphylactic shock, life-threatening reactions, sudden death) have been described. Asthma has a significantly higher association with peanut **allergy** than with other **allergies**, taken overall. As with other food **allergies**, diagnosis is based on history, prick-tests, screening for specific serum IgE and food challenge whose modalities (labial and oral challenge) are debated. For the time being, elimination is the only form of **treatment.** The development of a **modified allergen** as immunogenic as possible but practically without

allergenic effects should give immunotherapy new impetus. Patients with severe peanut **allergy** should carry a card or wear a distinctive bracelet indicating their condition as well as an emergency kit including in particular epinephrine. .COPYRGT. 2001 Editions scientifiques et medicales Elsevier SAS.

L4 ANSWER 15 OF 48 CAPLUS COPYRIGHT 2004 ACS on STN
2001:370988 Document No. 135:59830 Bypassing IgE and targeting T cells for specific immunotherapy of **allergy.** Akdis, Cezmi A.; Blaser, Kurt (Swiss Institute of Allergy and Asthma Research (SIAF), Davos, CH-7270, Switz.). Trends in Immunology, 22(4), 175-178 (English) 2001. CODEN: TIRMAE. ISSN: 1471-4906. Publisher: Elsevier Science Ltd..
AB A review with 22 refs. Specific immunotherapy (SIT) is a common **treatment** for allergic diseases. Despite its usage in clin. practice for nearly a century, more-rational and safer allergen prepns. are required. Here, the underlying mechanisms and principles of allergen modification for the future use of SIT in the **treatment** of **allergy** are discussed.

L4 ANSWER 16 OF 48 MEDLINE on STN DUPLICATE 8
2001262411. PubMed ID: 11306930. Engineering, characterization and in vitro efficacy of the major peanut allergens for use in immunotherapy. Bannon G A; Cockrell G; Connaughton C; West C M; Helm R; Stanley J S; King N; Rabjohn P; Sampson H A; Burks A W. (Department of Biochemistry and Molecular Biology, Arkansas Children's Hospital Research Institute, Little Rock 72205, USA.. bannongarya@exchnage.uams.edu) . International archives of allergy and immunology, (2001 Jan-Mar) 124 (1-3) 70-2. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.
AB BACKGROUND: Numerous strategies have been proposed for the **treatment** of peanut **allergies**, but despite the steady advancement in our understanding of atopic immune responses and the increasing number of deaths each year from peanut anaphylaxis, there is still no safe, effective, specific therapy for the peanut-sensitive individual. Immunotherapy would be safer and more effective if the allergens could be altered to reduce their ability to initiate an allergic reaction without altering their ability to desensitize the allergic patient. METHODS: The cDNA clones for three major peanut allergens, Ara h 1, Ara h 2, and Ara h 3, have been cloned and characterized. The IgE-binding epitopes of each of these allergens have been determined and amino acids critical to each epitope identified. Site-directed mutagenesis of the allergen cDNA clones, followed by recombinant production of the **modified allergen**, provided the reagents necessary to test our hypothesis that hypoallergenic proteins are effective immunotherapeutic reagents for treating peanut-sensitive patients. Modified peanut allergens were subjected to immunoblot analysis using peanut-positive patient sera IgE, T cell proliferation assays, and tested in a murine model of peanut anaphylaxis. RESULTS: In general, the **modified allergens** were poor competitors for binding of peanut-specific IgE when compared to their wild-type counterpart. The **modified allergens** demonstrated a greatly reduced IgE-binding capacity when individual patient serum IgE was compared to the binding capacity of the wild-type allergens. In addition, while there was considerable variability between patients, the **modified allergens** retained the ability to stimulate T cell proliferation. CONCLUSIONS: These **modified allergen** genes and proteins should provide a safe immunotherapeutic agent for the **treatment** of peanut **allergy.**
Copyright 2001 S. Karger AG, Basel

L4 ANSWER 17 OF 48 MEDLINE on STN DUPLICATE 9
2000290931. PubMed ID: 10828716. Regulation of specific immune responses by chemical and structural modifications of allergens. Akdis C A; Blaser K. (Swiss Institute of Allergy and Asthma Research (SIAF), Davos, Switzerland.. akdisac@siaf.unizh.ch) . International archives of allergy and immunology, (2000 Apr) 121 (4) 261-9. Ref: 103. Journal code:

9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB Specific immunotherapy (SIT) is an efficient **treatment** of allergic diseases to defined allergens. Despite being used in clinical practice since early in this century, more rational and safer regimens are required, because SIT is faced with the risk of anaphylaxis and standardization problems of allergen-extract-based **treatments.** A better understanding of the pathogenesis of **allergy** and of the mechanisms of SIT has led to various approaches to overcome these problems. Knowledge of the influence of IgE-facilitated antigen presentation on allergen-specific Th2 responses increased the efforts to generate non-IgE-binding allergens. The current principal approach to allergen modification is to modify B cell epitopes in order to prevent IgE binding and effector cell cross-linking while preserving T cell epitopes to retain the capacity of inducing tolerance. In this way, the **modified allergen** will be directed to T cells by a phagocytosis/pinocytosis-mediated antigen uptake mechanism, bypassing IgE cross-linking and IgE-dependent antigen presentation. Accordingly, a differential regulation of allergen-specific T cell cytokine patterns and IgE:IgG production was demonstrated by modifications of the three-dimensional structure of allergens because of linearity in T cell epitopes and conformation dependence in B cell epitopes. In this context, chemically **modified allergen** extracts with low IgE-binding capacity have been developed to reduce anaphylactic side effects since the early 1980s. The progress of recombinant techniques for producing allergens and allergen derivatives has led to a dramatic improvement in the ability of developing novel vaccines for the **treatment** of **allergy.** This has enabled mutation or deletion of decisive amino acids in B cell epitopes and fractionation or oligomerization of allergens by genetic engineering as fruitful approaches to generate hypoallergenic vaccines. Moreover, non-IgE-binding short T cell epitope peptides and single-amino-acid-altered peptide ligands represent potential candidates for future SIT.


L4 ANSWER 18 OF 48 EMBASE 
on STN DUPLICATE 10

2000172489 EMBASE [Therapeutic **allergy** vaccination - Causal **treatment** of pollen **allergy**, part II: Clinical aspects of **allergy** vaccination]. THERAPEUTISCHE ALLERGIEIMPFUNG - KAUSALE BEHANDLUNG DER POLLENALLERGIE TEIL II: KLINISCHE ASPEKTE DER ALLERGIEIMPFUNG. Wolf H.; Van Neerven R.J.J.; Larsen J.N.; Spangfort M.D.; Lowenstein H.. Dr. H. Wolf, ALK-SCHERAX Arzneimittel GmbH, Sulldorfer Landstrasse 128, D-22589 Hamburg, Germany. Allergologie 23/4 (182-189) 2000.
Refs: 37.
ISSN: 0344-5062. CODEN: ALLRDI. Pub. Country: Germany. Language: German. Summary Language: English; German.

AB Specific **allergy** vaccination against seasonal allergens is used today either as a perennial therapy over three years or as a preseasonal short-term therapy. The allergen extracts used are composed of intact or chemically **modified allergens.** The largest effects in placebo-controlled, double-blind clinical trials have been observed for the perennial **allergy** vaccination with intact allergens. Lower effects have been described for the preseasonal short-term **treatment** with intact allergens and for the perennial **treatment** with chemically **modified allergens.** No data from placebo-controlled, double- blind studies have been published for short-term **treatment** with chemically **modified allergens** so far. There is some evidence that **allergy** vaccination has a preventive effect against asthma. As a mechanism of the efficacy a shift from a TH2-T helper cell cytokine profile to a TH1 cytokine profile is postulated. The decreased immigration of effector cells like eosinophils results in a reduced late-phase reaction.

L4 ANSWER 19 OF 48 BIOSIS  on STN

2001:30533 Document No.: PREV200100030533. Structure and biology of stinging insect venom allergens. King, Te Piao [Reprint author]; Spangfort, Michael D.. Rockefeller University, 1230 York Avenue, New York, NY, 10021-6399, USA. International Archives of Allergy and Immunology, (October, 2000) Vol. 123, No. 2, pp. 99-106. print.
CODEN: IAAIEG. ISSN: 1018-2438. Language: English.

AB Bees, fire ants and vespids cause insect sting **allergy.** These insects have unique as well as common venom allergens. Vespids, including hornets, paper wasps and yellow jackets, have common allergens. Bees and vespids have one common allergen with hyaluronidase activity; they also have unique allergens with different phospholipase activities. Fire ants and vespids have one common allergen, antigen 5 of unknown biologic activity. The common venom allergens with < 70% sequence identity have barely detectable levels of antigenic cross-reactivity. Possible uses of **modified allergens** for immunotherapy are described.

L4 ANSWER 20 OF 48 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 11

2000081736 EMBASE [Therapeutic **allergy** vaccination causal **treatment** of pollen **allergy.** Part I: Allergen complexity and standardization]. THERAPEUTISCHE ALLERGIEIMPFUNG: KAUSALE BEHANDLUNG DER POLLENALLERGIE TEIL I: ALLERGENKOMPLEXITAT UND STANDARDISIERUNG. Larsen J.N.; Lowenstein H.; Van Neerven R.J.J.; Spangfort M.D.; Wolf H.. Dr. H. Wolf, ALK-SCHERAX Arzneimittel GmbH, Sulldorfer Landstrasse 128, D-22589 Hamburg, Germany. Allergologie 23/2 (92-96) 2000.
Refs: 10.
ISSN: 0344-5062. CODEN: ALLRDI. Pub. Country: Germany. Language: German. Summary Language: English; German.

AB The WHO immunotherapy position paper on allergen immunotherapy published in June, 1998, introduces the term 'vaccine' instead of 'allergen extract'. This fact may be regarded a recognition of the level of modern standardization reflecting the continuing refinement of allergen technology. Standardization of allergen vaccines includes the exact characterization of the allergen content, the quantification of the major allergens and a constant allergenic potency. Allergen vaccines are applied for the **treatment** of patients as aqueous or depot preparations of intact or chemically **modified allergens.** While all epitopes are conserved in intact allergen vaccines the chemical modification induces a selective destruction of lysine-containing epitopes resulting in a larger patient to patient variation of the response to the allergen vaccine.

L4 ANSWER 21 OF 48 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2000202519 EMBASE [Standardisation of tyrosine-adsorbed glutaraldehyde - Modified grass pollen extract (Pollinex® Grass)]. STANDARDIZACIA EXTRAKTOV TRAVNYCH PEL'OV ADSORBOVANYCH NA TYROZIN A MODIFIKOVANYCH GLUTARALDEHYDOM (POLLINEX® GRASS). Wheeler A.W.; Lees B.. A.W. Wheeler, Allergy Therapeutics Ltd., Dominion Way, Worthing, West Sussex BN14 8SA, United Kingdom. Klinicka Imunologia a Alergologia 10/1 (38-42) 2000.
Refs: 6.
ISSN: 1335-0013. CODEN: KIALEZ. Pub. Country: Slovakia. Language: Slovak. Summary Language: English; Slovak.

AB Allergen immunotherapy using a standardised **modified allergen** extracts has been used for successful **treatment** of type I **allergy** for many years. All constituents of **allergy** vaccines are subject of analysis to improve the standardisation of vaccines over last decade. Method of new approaches for this shows this impartion.

L4 ANSWER 22 OF 48 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

1999237932 EMBASE Mutant derivatives of the main respiratory allergen of cow are less allergenic than the intact molecule. Kauppinen J.; Zeiler T.;

Rautiainen J.; Rytkonen-Nissinen M.; Taivainen A.; Mantyjarvi R.; Virtanen T.. T. Virtanen, Department of Clinical Microbiology, University of Kuopio, POB 1627, FIN-70211 Kuopio, Finland. Clinical and Experimental Allergy 29/7 (989-996) 1999.
Refs: 35.
ISSN: 0954-7894. CODEN: CLEAEN. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB Background Allergen immunotherapy offers an alternative for drug **treatment** in the management of allergic diseases. Because immunotherapy often induces side-effects, less allergenic preparations would be beneficial. Objective The purpose of this study was to examine whether the allergenicity of a cow-derived lipocalin allergen, Bos d 2, could be diminished by substituting or deleting carboxy-terminal amino acids including the cysteine which forms a disulphide bond with a cysteine inside the molecule. Methods Four recombinant mutants of Bos d 2 were created by substituting or deleting the four most carboxy-terminal amino acids. The immunological characteristics of the mutant preparations were compared with the unmodified rBos d 2 by Western blotting, ELISA inhibition, skin prick tests, and the proliferative responses of allergen-specific T-cell clones. Results In Western blot, one of the two monoclonal antibodies showed reduced binding to the preparations without the terminal cysteine. In contrast, the other monoclonal antibody, human IgE and rabbit immune serum bound equally well to all the preparations. ELISA inhibition analyses revealed, however, that the preparations without the terminal cysteine bound antibody less efficiently. They were needed 15- 38 times more than the unmodified rBos d 2 to cause the same level of inhibition. Surprisingly, one of the mutants with the terminal cysteine but a mutated adjacent amino acid turned out to be the weakest in inducing skin reactivity. All the preparations stimulated well allergen-specific T-cell clones. Conclusions The results show that the allergenicity of a lipocalin allergen, Bos d 2, can be diminished by modifying the carboxy-terminal end of the molecule. Modifications in the area which encompasses a disulphide bond impaired the antibody binding without affecting the T-cell stimulatory capacity. It was also shown that in vivo tests are necessary for determining the allergenicity of a **modified allergen.**

L4 ANSWER 23 OF 48 CAPLUS COPYRIGHT 2004 ACS on STN
1999:56260 Document No. 130:266025 Vaccines against **allergies.**
Hellman, L. (Department of Medical Immunology and Microbiology, BMC, Uppsala, S-751 23, Swed.). Handbook of Experimental Pharmacology, 133(Vaccines), 499-526 (English) 1999. CODEN: HEPHD2. ISSN: 0171-2004. Publisher: Springer-Verlag.

AB A review with 135 refs. A detailed description of allergic immune response along with a discussion of some of the currently available immunotherapies is presented. Application of **modified allergens**, oral administration of allergens and allergen exts., peptide vaccines, cytokine agonists and antagonists as immunotherapeutic approach is discussed. In addition, use of low mol. weight substances that interfere with the interactions between IgE and its receptors as well as strategies involving the depletion of plasma and mast cell bound IgE by **treatment** with monoclonal anti-IgE antibodies is also mentioned. Finally, strategies involving induction of strong anti-IgE response by vaccination are outlined.

L4 ANSWER 24 OF 48 MEDLINE on STN DUPLICATE 12
1999297499. PubMed ID: 10371089. Long-term **treatment** with allergoid immunotherapy with Parietaria. Clinical and immunologic effects in a randomized, controlled trial. Ariano R; Kroon A M; Augeri G; Canonica G W; Passalacqua G. (Servizio di Allergologia, Ospedale di Bordighera (IM), Italy. ) Allergy, (1999 Apr) 54 (4) 313-9. Journal code: 7804028. ISSN: 0105-4538. Pub. country: Denmark. Language: English.

AB BACKGROUND: Specific immunotherapy (SIT) is a valuable **treatment** for respiratory **allergy**, and the use of chemically **modified allergens** (allergoids) has improved its safety,

as testified by several studies. We evaluated the effects of a SIT course with an allergoid extract of Parietaria pollen in a double-blind, placebo-controlled trial. METHODS: The study was double-blind in the first year; then it was prolonged up to 3 years with all patients on active **treatment.** Clinical effectiveness, safety, skin reactivity, systemic immunologic parameters, and subjective assessment were evaluated. We also had available a self-evaluation recorded in a follow-up visit 4 years after the discontinuation of SIT. RESULTS: A significant reduction of the symptoms plus drug intake scores during the pollen seasons was observed in the patients receiving active SIT. The placebo patients, after switching to active SIT, also showed significant clinical improvement. The clinical efficacy persisted during years 2 and 3 of **treatment.** After year 1, the actively treated patients reported a significant subjective improvement (frequency of symptoms, P = 0.001; duration of symptoms, P = 0.024; physical performance, P = 0.043) compared with the placebo group. The self-evaluation by visual analog scale showed that all patients maintained a significant clinical improvement up to 4 years after discontinuing SIT (year 1: active=+31.6%, placebo=-15.7%; year 7: active=+35.8%, placebo=+31.3%). The systemic immunologic changes after active SIT paralleled those described elsewhere (IgE decreased from 22 to 9 and from 21 to 8 IU/ml; IgG4 increased from 43 to 87 and from 18 to 60 IU/ml). A significant decrease in skin reactivity to three different allergen concentrations was observed at year 3 compared with pretreatment values (P<0.05). CONCLUSIONS: The investigational SIT with Parietaria appeared to be effective and safe; a 3-year course of **treatment** achieved a long-lasting efficacy.

L4 ANSWER 25 OF 48 EMBASE 
on STN

1999307042 EMBASE Future prospects for immunotherapy. Frew A.J.. Dr. A.J. Frew, University Medicine (MP 810), Southampton General Hospital, Southampton SO1 6YD, United Kingdom. A.J.Frew@soton.ac.uk. International Review of Allergology and Clinical Immunology 5/3 (193-197) 1999. Refs: 26.
ISSN: 1232-9142. CODEN: IRAIFY. Pub. Country: Poland. Language: English. Summary Language: English; Polish.

AB Focus on improving the safety profile of specific immunotherapy by using **modified allergens**, recombinanat allergens DNA and peptide vaccines indicate the future prospects for these **treatment** of allergic diseases.

L4 ANSWER 26 OF 48 CAPLUS 

1998:682305 Document No. 129:321186 Allergen formulations containing tyrosine and deacetylated monophosphorylated lipid A. Ulrich, Jorj Terry; Wheeler, Alan Worland (Smithkline Beecham Plc, UK). PCT Int. Appl. WO 9844947 A1 19981015, 11 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-EP2138 19980403. PRIORITY: GB 1997-6957 19970405.

AB A pharmaceutical composition comprises tyrosine, an optionally **modified allergen**, and 3-DMPL (3-de-O-acylated lipid A monophosphorylated), is useful in the prevention and **treatment** of **allergy.** Thus, a 0.5 mg/mL grass pollen extract was modified by **treatment** with 0.25% glutaraldehyde. A pH 7 phosphate buffer was added and the allergen solution was copptd. with tyrosine by the simultaneous addition of L-tyrosine and 3.2M NaOH. A DPPC solution was mixed with 3-DMPL and combine with tyrosine.

L4 ANSWER 27 OF 48 EMBASE 
on STN DUPLICATE 13

1998417846 EMBASE Responses of human birch pollen allergen-reactive T cells to chemically **modified allergens** (allergoids). Dormann D.; Ebner C.; Jarman E.R.; Montermann E.; Kraft D.; Reske-Kunz A.B.. Dr. A.B. Reske-Kunz, Klinische Forschergruppe Allergie, Univ.-Hautklinik/Verfugungsgebaude, Obere Zahlbacher Strasse 63, D-55131 Mainz, Germany. Clinical and Experimental Allergy 28/11 (1374-1383) 1998. Refs: 29.
ISSN: 0954-7894. CODEN: CLEAEN. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB Background: Allergoids are widely used in specific immunotherapy for the **treatment** of IgE-mediated allergic diseases. Objective: The aim of this study was to analyse whether a modification of birch pollen allergens with formaldehyde affects the availability of T-cell epitopes. Methods: Efficient modification of the allergens was verified by determining IgE and IgG binding activity using ELISA inhibition tests. T-cell responses to birch pollen allergoids were analysed in polyclonal systems, using peripheral blood mononuclear cells (PBMC) of five birch pollen-allergic individuals, as well as birch pollen extract-reactive T-cell lines (TCL), established from the peripheral blood of 14 birch pollen-allergic donors. To determine whether the modification of natural (n)Bet v 1 with formaldehyde or maleic anhydride results in epitope-specific changes in T-cell reactivities, 22 Bet v 1- specific T-cell clones (TCC), established from nine additional birch pollen- allergic individuals, were tested for their reactivity with these products. Results: The majority of PBMC and TCL showed a reduced response to the birch pollen extract allergoid. Bet v 1-specific TCC could be divided into allergoid-reactive and -non-reactive TCC. No simple correlation between possible modification sites of formaldehyde in the respective T-cell epitopes and the stimulatory potential of the allergoid was observed. Mechanisms of suppression or of anergy reduction were excluded as an explanation for the non-reactivity of representative TCC. All TCC could be stimulated by maleylated and unmodified nBet v 1 to a similar extent. Conclusion: These results demonstrate differences in the availability of T-cell epitopes between allergoids and unmodified allergens, which are most likely due to structural changes within the allergen molecule.

L4 ANSWER 28 OF 48 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1999:43440 Document No.: PREV199900043440. Long-term analysis of allergen-specific T cell clones from patients with asthma treated with allergen rush immunotherapy. Oda, Naruhito [Reprint author]; Yamashita, Naomi; Minoguchi, Kenji [Reprint author]; Takeno, Mitsuhiro; Kaneko, Sakae; Sakane, Tsuyoshi; Adachi, Mitsuru [Reprint author]. First Dep. Internal Med., Showa Univ., Tokyo, Japan. Cellular Immunology, (Nov. 25, 1998) Vol. 190, No. 1, pp. 43-50. print.
CODEN: CLIMB8. ISSN: 0008-8749. Language: English.

AB Rush immunotherapy (RI), a **modified allergen**-specific immunotherapeutic procedure, is an effective **treatment** for extrinsic (atopic) asthma, although the precise mechanism of its action is unclear. We have thus investigated the effect of RI on T cell response in seven mite-allergen-sensitive asthmatic patients who were successfully treated with RI. The proliferative response to mite allergen profoundly decreased after 3 months of therapy compared to the response before therapy; the response, however, recovered 18 months after RI. Regarding cytokine production patterns of mite-specific T cells, RI brought about a shift in cytokine profiles from Th2 to Th0 or Th1 in mite-specific T cell clones. The data indicate that the efficacy of RI is due to modification of T cell responses to mite antigens. Allergen RI results in the conversion of Th2 to Th1 and Th0 cells and/or selection of Th1 and Th0 cells over Th2 cells and thus may improve both clinical symptoms and airway inflammation in asthmatics.

L4 ANSWER 29 OF 48 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
97159318 EMBASE Document No.: 1997159318. Immunotherapy for insect **allergy**: Recent advances and therapeutic perspectives. Muller

U.R.. U.R. Muller, Medical Division, Zieglerspital, CH-3000 Bern, Switzerland. International Journal of Immunopathology and Pharmacology 10/2 SUPPL. (155-156) 1997.
ISSN: 0394-6320. CODEN: IJIPE4. Pub. Country: Italy. Language: English. Summary Language: English.

AB Indications for venom immunotherapy (VIT) are still somewhat controversial. While most allergists propose VIT in the presence of a history of severe systemic allergic reactions following hymenoptera stings and positive diagnostic tests, Dutch authors propose the diagnostic sting challenge as a selection criterion to start VIT. Considerable new data regarding the required duration of VIT have been accumulated during the last years, indicating that after 3 - 5 years of **treatment**, long-term protection even after stopping **treatment** can be expected in more than 80 - 90 % of the patients. Because VIT, especially with honey bee venom induces systemic allergic side effects frequently, modifications of this **treatment** have been proposed. Pretreatment with antihistamines and the use of **modified allergens** is discussed. Immunotherapy with T cell epitopes of major venom allergens, although still experimental, is promising.

L4 ANSWER 30 OF 48 EMBASE 
on STN DUPLICATE 14

97052187 EMBASE Document No.: 1997052187. Characterization of allergoids from ovolbumin in vitro and in vivo. Salgado J.; Casadevall G.; Puignero V.; Queralt J.. Dr. J. Queralt, Unitat de Fisiologia, Facultat de Farmacia, Universitat de Barcelona, Joan XXIII sn, 08028 Barcelona, Spain. Immunobiology 196/4 (375-386) 1996.
Refs: 22.
ISSN: 0171-2985. CODEN: ZIMMDO. Pub. Country: Germany. Language: English. Summary Language: English.

AB Several in vivo and in vitro methods for monitoring immunological properties of two allergoids obtained by formaldehyde **treatment** of ovalbumin (OA) were developed. The calculated molecular weight of allergoids was 80 kD (OA-F1) and 165 kD (OA-F2), respectively. The allergenic activity in vitro of allergoids in mast-cell histamine release assay was 1000 times lower than of OA. Both allergoids showed reduced ability to induce passive cutaneous anaphylaxis in the Sprague-Dawley rats or systemic anaphylaxis in Dunkin-Harley guinea-pigs. The ability of OA and allergoids to bind to the OA-specific IgE antibodies was measured in vivo by the inhibition of passive cutaneous anaphylaxis (PCA-inhibition). Allergoid binding to IgE was 51-66% lower than the native allergen. Moreover, the avidity of OA-specific IgG antibodies, measured by ELISA-inhibition, for allergoids and allergen was of the same order. Allergoids induced a different pattern of humoral immune response from that, induced by the native allergen. Thus, after immunization of BALB/c mouse, both allergoids induced a higher production of IgG and a lower production of IgE than OA, only OA-F2 induced a lower production of IgG,. The differences in the IgA response to the immunogens was not significant. Delayed hypersensitivity studies in the BALB/c mouse showed that allergoids were 5- to 12-times less effective in inducing a cell-mediated immune response than OA. The present study provides a battery of immunological methods for preclinical testing of **modified allergens.**

L4 ANSWER 31 OF 48 MEDLINE on STN DUPLICATE 15

96068619. PubMed ID: 7478651. Future immunotherapy: what lies ahead?. Gordon B R. (Massachusetts Eye & Ear Infirmary, Boston, USA. ) Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery, (1995 Nov) 113 (5) 603-5. Journal code: 8508176. ISSN: 0194-5998. Pub. country: United States. Language: English.

AB There is currently great interest in developing improved methods of immunotherapy and new techniques of immune system manipulation to ameliorate allergic diseases. This article reviews current research trends in the immunologic **treatment** of **allergy**,

including the use of chemically **modified allergens**, nonparenteral allergen exposure, sustained-release allergen delivery, anti-immunoglobulin E antibodies, gamma-globulin, immune complexes, cytokines, and T-cell-tolerogenic peptides.

L4 ANSWER 32 OF 48 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1994:55457 Document No.: PREV199497068457. Allergen extracts of the future. Wahl, R.. Hermann-Koerner-Strasse 52, D-21465 Reinbek bei Hamburg, Germany. Allergologie, (1993) Vol. 16, No. 11, pp. 445-453. CODEN: ALLRDI. ISSN: 0344-5062. Language: German.

AB In addition to the expertise of the doctor and the cooperation of his patient, the successful diagnosis and therapy of allergic disease depend to a large extent on the quality of the allergen extracts. Many immunochemical methods are used for the characterisation and standardisation of allergen extracts which enable its composition to be defined on a molecular basis. In the near future it may be possible to quantify single allergens in these extracts by the use of the sandwich ELISA technique employing monoclonal antibodies. The future will also show whether or not recombinant allergens have a role to play in the diagnosis and **treatment** of allergic disease. However at the present time chemically **modified allergens**, termed allergoids, especially depot allergoids, provide one means of immunotherapy that has proved in clinical trials to be both efficacious and safe.

L4 ANSWER 33 OF 48 MEDLINE on STN DUPLICATE 16
93107727. PubMed ID: 7678032. Allergen-specific modulation of cytokine synthesis patterns and IgE responses in vivo with chemically **modified allergen.** Gieni R S; Yang X; HayGlass K T. (Department of Immunology, University of Manitoba, Winnipeg, Canada. ) Journal of immunology (Baltimore, Md. : 1950), (1993 Jan 1) 150 (1) 302-10. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Hypersensitivity and IgE synthesis are highly dependent on the balance in which production of IL-4 and IFN-gamma is induced. An immunologic approach that alters the dominant pattern of cytokine synthesis and antibody production that is elicited after exposure to native allergen is described. High M(r), glutaraldehyde-polymerized OVA administered (i.p.) before or after immunization with unmodified OVA induces > or = 95% inhibition of specific IgE synthesis concomitant with 300- to 800-fold increases in IgG2a production in C57BL/6 mice. These changes result from a genetically controlled shift in the pattern of cytokine production within the allergen-specific T cell repertoire as demonstrated by i) susceptibility of the changes induced upon administration of **modified allergen** to in vivo **treatment** with anti-IFN-gamma mAb and ii) a 5- to 7-fold increase in the ratio of IFN-gamma:IL-4 synthesis after overnight culture directly ex vivo. This system should prove useful in identification of the factors which are influential in the commitment of T cells to Th1- or Th2-like patterns of cytokine synthesis. Moreover, as defective induction of IFN-gamma by allergen-specific T cells appears to play a role in elevated IgE synthesis and human **allergy**, this approach may have therapeutic potential.

L4 ANSWER 34 OF 48 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
91016632 EMBASE Document No.: 1991016632. Isotype-selective abrogation of established IgE responses. HayGlass K.T.; Stefura W.. Department of Immunology, University of Manitoba, 730 William Avenue,Winnipeg, Man. R3E 0W3, Canada. Clinical and Experimental Immunology 82/3 (429-434) 1990. ISSN: 0009-9104. CODEN: CEXIAL. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB Chemically **modified allergens** have been extensively studied in an attempt to develop materials of increased efficacy and improved safety for use in the immunotherapy of allergic disease. Most of the strategies that have been developed yield products that strongly

inhibit de novo IgE responses but have only marginal impact on ongoing IgE responses. We report the virtual abrogation of pre-established murine anti-ovalbumin IgE responses using a glutaraldehyde-polymerized ovalbumin preparation (OA-POL) of M(r) 3.5 x 107. Secondary IgE responses are inhibited by 97-99% over a period of at least 8 months following three i.p. courses of OA-POL **treatment.** Administration of five additional ovalbumin [Al(OH)3] booster immunizations over this period fails to alter this unresponsive state. The inhibition of antigen-specific IgE responses is isotype specific.

L4 ANSWER 35 OF 48 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1991:95234 Document No.: PREV199191054124; BA91:54124. INVESTIGATIONS ON PARIETARIA ALLERGOID IMMUNOTHERAPY II. IMMUNOLOGICAL MODIFICATIONS UNDER IMMUNOTHERAPY WITH UNMODIFIED AND **MODIFIED ALLERGENS** FROM PARIETARIA-JUDAICA. BRUNO G [Reprint author]; MARI A; FRANK E; FRANCESCHINI P; BIOCCA M M; BALSANO F. ISTITUTO DI I CLINICA MEDICA GENERALE TERAPIA MEDICA, POLICLINICO UMBERTO I, UNIV DEGLI STUDI DI ROMA, LA SAPIENZA, I-00161 ROMA. Allergologie, (1990) Vol. 13, No. 11, pp. 424-432.
CODEN: ALLRDI. ISSN: 0344-5062. Language: GERMAN.

AB To study safety and efficacy of an alum-adsorbed Parietaria pollen allergoid a prospective clinical trial was performed including 39 Parietaria pollen allergic patients: 15 patients were treated with an allergoid depot extract, 16 patients with a conventional allergen depot preparation and 8 patients, who served as control group, only received symptomatic medication during the pollen season. Detailed results on safety and clinical efficacy were reported separately [3], immunological investigations are subject of this report. Investigations on the extent and influence of changes of immunological parameters demonstrated a significant ($p < 0.001$) reduction of the skin reactivity after hyposensitization, especially after **treatment** with the **modified allergen.** Only for this allergoid therapy we found an additional significant ($p < 0.01$) correlation between the reduction of the skin reactivity and the increase of specific IgG (sIgG) values after therapy. Whereas no relevant changes of sIgG were seen in the control group, significant ($p < 0.001$) increases were registered after therapy for the allergoid group with a net increase of 345% and for the allergen group of 132%. No significant changes of lymphocyte values (T3, T4, T8, T4/T8) were found within the 3 trial groups over the study period of 1 year. Summarizing we can say that this study did not only result in a clear advantage for the hyposensitized patients in comparison to the control group but the results also indicate a further advance of the group treated with the **modified allergen** extract with a reduced allergenicity and yet an excellent immunogenicity, thus reducing the number of injections. On the basis of the successful results found for immunotherapy with the depot allergoid extract it could be useful to continue research in this direction.

L4 ANSWER 36 OF 48 MEDLINE on STN
91179766. PubMed ID: 2080061. [Hyposensitization: indications and expectations]. Hyposensibilisierung: Indikation und Erwartungen. Urbanek R. (Universitats-Kinderklinik Freiburg, Bundesrepublik Deutschland. ) Padiatrie und Padologie, (1990) 25 (6) 397-404. Ref: 13. Journal code: 0022370. ISSN: 0030-9338. Pub. country: Austria. Language: German.

AB **Allergy** is the most frequent immunologic disorder in childhood. The prevalence of allergic complaints among children is estimated as about 10%. The diagnosis of an **allergy** takes the following factors into account: The patient's history, skin test, determination of total and specific IgE antibodies and a provocation test to a diseased organ. Hyposensitization is recommended for allergic patients without any significant improvement in spite of avoidance of allergen and in spite of pharmacologic therapy. **Modified allergens**/allergoids demonstrate a comparable efficacy as a conventional subcutaneous allergen immunotherapy. Measurement of specific IgE- and IgG-antibodies permits an evaluation of degree of sensitization and/or immune response to

hyposensitization **treatment.**

L4 ANSWER 37 OF 48 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1990:151435 Document No.: PREV199089078853; BA89:78853. DEMONSTRATION OF HUMAN RYE GRASS POLLEN EXTRACT-SPECIFIC HELPER AND SUPPRESSOR STIMULATING ACTIVITY OF **MODIFIED ALLERGENS** BY EFFECTS ON IN-VITRO HAPTEN-SPECIFIC ANTIBODY PRODUCTION. WHEELER A W [Reprint author]; JOHANSSON S G O. BENCARD ALLERGY UNIT, BEECHAM PHARM GREAT BURGH, YEW TREE BOTTOM RD, EPSOM, SURREY KT18 5XQ, UK. International Archives of Allergy and Applied Immunology, (1989) Vol. 90, No. 4, pp. 320-325.
CODEN: IAAAAM. ISSN: 0020-5915. Language: ENGLISH.
AB An in vivo system is described in which penicilloyl antibody was produced from peripheral leucocytes of a grass pollen-sensitive patient who had received penicillin therapy, by challenge of the cells with penicilloyl-grass pollen extract conjugate. Incubation of these leucocytes with a number of modified preparations of grass pollen extract with various T-cell-stimulating properties was shown to affect penicilloyl antibody production. Both chymotryptically fragmented rye grass pollen extract and a conjugate of met-leu-phe and rye grass pollen extract enhanced penicilloyl-specific antibody similarly to the enhancement induced by unmodified extract, though at high concentration some suppression was seen. A conjugate of polysarcosine and rye grass pollen extract, previously shown to cause antibody suppression in mice, was similarly suppressive for penicilloyl-specific antibody. The system therefore shows potential for the evaluation of the effects of **modified allergen treatment** on antibody levels via T-cell mechanisms.

L4 ANSWER 38 OF 48 CAPLUS COPYRIGHT 2004 ACS on STN
1989:475957 Document No. 111:75957 Improvement of specific immunotherapy by human IgG and **modified allergens.** Poulsen, Lars K.; Soendergaard, I.; Weeke, B. (Med. Dep. TTA, State Univ. Hosp., Copenhagen, DK-2200, Den.). Allergy (Oxford, United Kingdom), 44(4), 241-55 (English) 1989. CODEN: LLRGDY. ISSN: 0105-4538.
AB A review with 131 refs.

L4 ANSWER 39 OF 48 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
88239955 EMBASE Document No.: 1988239955. Allergic rhinitis: Recent advances. Simons F.E.R.. Section of Allergy and Clinical Immunology, Department of Pediatrics and Child Health, Faculty of Medicine, University of Manitoba, Winnipeg, Man., Canada. Pediatric Clinics of North America 35/5 (1053-1074) 1988.
ISSN: 0031-3955. CODEN: PCNAA8. Pub. Country: United States. Language: English. Summary Language: English.
AB Our understanding of the pathophysiology of allergic rhinitis is increasing as a result of the development of new research tools for this investigation of patients with this disorder. The pharmacologic **treatment** of allergic rhinitis has been greatly improved by introduction of relatively nonsedating H1-receptor antagonists and potent, topically active, glucocorticosteroids. Immunotherapy for allergic rhinitis is also changing with the times. Patient selection criteria are becoming more strict, and the introduction of safer, **modified allergens** with decreased allergenicity and retained immunogenicity will be a major advance.

L4 ANSWER 40 OF 48 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1985:366842 Document No.: PREV198580036834; BA80:36834. RECOGNITION AND **TREATMENT** OF ALLERGIC DISORDERS IN CHILDREN. URBANEK R [Reprint author]; KUHN W. MATHILDENSTRASSE 1, D-7800 FREIBURG I BR. Allergologie, (1985) Vol. 8, No. 5, pp. 206-209.
CODEN: ALLRDI. ISSN: 0344-5062. Language: GERMAN.
AB **Allergy** is the most frequent immunologic disorder in childhood. The prevalence of allergic complaints among children is .apprx. 10%. **Allergy** diagnosis takes into account patient history, skin test,

determination of total and specific IgE antibodies and a provocation test to a diseased organ. Conjunctival provocation, bronchial challenge and rhinomanometry are the most used provocation procedures. Hyposensitization is recommended for allergic patients without any significant improvement in spite of avoidance of allergen and in spite of pharmacologic therapy. **Modified allergens**/allergoids demonstrate a comparable efficacy was a conventional s.c. allergen immunotherapy. Regular measurement of specific IgE and IgG antibodies permits an evaluation of the degree of sensitization and/or protection from allergic symptoms.

L4 ANSWER 41 OF 48 MEDLINE on STN DUPLICATE 17
84160696. PubMed ID: 6706424. Polyethylene glycol reactive antibodies in man: titer distribution in allergic patients treated with monomethoxy polyethylene glycol **modified allergens** or placebo, and in healthy blood donors. Richter A W; Akerblom E. International archives of allergy and applied immunology, (1984) 74 (1) 36-9. Journal code: 0404561. ISSN: 0020-5915. Pub. country: Switzerland. Language: English.
AB Antibodies to polyethylene glycol (PEG) were analyzed in patients with various **allergies** and in healthy blood donors employing passive hemagglutination. In untreated allergic patients and in healthy blood donors, naturally occurring anti-PEG antibody titers between 32 and 512 were seen in 3.3 and 0.2%, respectively. During hyposensitization with monomethoxy polyethylene glycol modified ragweed extract and honey bee venom, respectively, the patients showed an anti-PEG antibody response. Titers of 32-512 were found in 50% of the patients directly after the first **treatment** course. After 2 years of **treatment** the percentage of patients with such titers declined to 28.5%. Mercaptoethanol **treatment** of sera indicated that the anti-PEG antibodies predominantly were of the IgM isotype. The weak IgM response found in treated patients is considered to be of no clinical significance.

L4 ANSWER 42 OF 48 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1985:100941 Document No.: PREV198528100941; BR28:100941. PROSPECTS IN THE APPLICATION OF NEW **MODIFIED ALLERGENS** FOR THE **TREATMENT** OF ATYPICAL ALLERGIC DISEASES. RAIKIS B N [Reprint author]; VORONKIN N I; GERVAZIEVA V B. RES INST VACC SERA, STAVROPOL, USSR. Immunologiya, (1984) No. 4, pp. 12-17. ISSN: 0206-4952. Language: RUSSIAN.

L4 ANSWER 43 OF 48 MEDLINE on STN DUPLICATE 18
84121212. PubMed ID: 6229874. [Immunotherapy of allergic diseases: present and future]. Immuntherapie allergischer Krankheiten: Gegenwart und Zukunft. Muller U. Schweizerische medizinische Wochenschrift, (1983 Dec 31) 113 (52) 1982-8. Ref: 38. Journal code: 0404401. ISSN: 0036-7672. Pub. country: Switzerland. Language: German.
AB The efficacy of immunotherapy (IT) of immediate type **allergies** is well established but its mode of action is unclear. Indication for IT is based on history confirmed by skin tests, RAST and possibly provocation tests. Nature, severity and duration of allergic symptoms must be considered. IT is especially suitable as a basic **treatment** of seasonal allergic rhinitis and pollen asthma as well as perennial rhinitis and asthma due to the house dust mite. The value of IT with molds and bacterial vaccines is questionable. With insect venoms IT is well established as a means of preventing anaphylactic reactions to hymenoptera stings. Improvement of IT will be brought about chiefly by better standardization and purification of allergen extracts. The significance of IT with chemically **modified allergens** and using different modes of application (oral, nasal) cannot be definitely evaluated at present.

L4 ANSWER 44 OF 48 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1983:281217 Document No.: PREV198376038709; BA76:38709. ANTIGEN SPECIFIC AND IMMUNO GLOBULIN E CLASS SPECIFIC SUPPRESSION MEDIATED BY T SUPPRESSOR CELLS OF MICE TREATED WITH GLUTARALDEHYDE POLYMERIZED OV ALBUMIN. HAYGLASS

K T [Reprint author]; STREJAN G H. DEP MICROBIOL AND IMMUNOL, UNIV WESTERN ONT, LONDON, ONT N6A 5C1, CAN. International Archives of Allergy and Applied Immunology, (1983) Vol. 71, No. 1, pp. 23-31.
CODEN: IAAAAM. ISSN: 0020-5915. Language: ENGLISH.

AB Various size polymers [POL] are obtained following glutaraldehyde **treatment** of native ovalbumin (OA). OA-POL, .apprx. 35 + 106 daltons, was prepared at the isoelectric point of OA. **Treatment** of CBA mice with microgram amounts of OA-POL led to efficient antigen-specific suppression of IgE responses. IgG anti-OA antibodies were not suppressed. Transfer of cells from OA-POL-treated donors into normal, unprimed recipients interfered with the ability of these animals to mount a primary or secondary IgE response. Cotransfer of spleen cells from OA-POL-treated mice along with OA (in alum)-primed clels, into irradiated syngeneic recipients resulted in IgE class-specific suppression that was abrogated by **treatment** of OA-POL donor cells with monoclonal anti-Thy 1.2 + complement. The presence or absence of T cells in the OA-POL population had no effect on IgG levels in the recipients. Analysis of the antigenic properties of OA-POL revealed 5-15% cross-reactivity with native OA as perceived by IgG or IgE antibodies. OA-POL was highly cross-reactive at the T cell level as shown functionally by its potent induction of OA-specific, IgE-selective suppressor T cells. The beneficial effects of glutaraldehyde-**modified allergens**, recently introduced in the immunotherapy of atopic individuals may be due to the preferential exposure on the polymerized protein, of antigenic determinants generating T suppressor cells and to the selective loss of B cell-reactive determinants.

L4 ANSWER 45 OF 48 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1981:220147 Document No.: PREV198172005131; BA72:5131. SUPPRESSION OF REAGINIC ANTIBODIES WITH **MODIFIED ALLERGENS** 3. PREPARATION OF TOLEROGENIC CONJUGATES OF COMMON ALLERGENS WITH MONO METHOXY POLY ETHYLENE GLYCOLS OF DIFFERENT MOLECULAR WEIGHTS BY THE MIXED ANHYDRIDE METHOD. WIE S I [Reprint author]; WIE C W; LEE W Y; FILION L G; SEHON A H; AKERBLOM E. DEP OF IMMUNOL, UNIV OF MANITOBA, BASIC MED SCI BLDG, WINNIPEG, MANITOBA R3E 0W3, CANADA. International Archives of Allergy and Applied Immunology, (1981) Vol. 64, No. 1, pp. 84-99.
CODEN: IAAAAM. ISSN: 0020-5915. Language: ENGLISH.

AB Antigens, such as ovalbumin (OA) and the extract of ragweed pollen (RAG), could be rendered nonantigenic, nonallergenic and tolerogenic by conjugation with polyethylene glycol (PEG). The synthesis of conjugates of a variety of antigens with monofunctional monomethoxy-PEG (mPEG) of different MW by the use of the mixed anhydride method. Thus, mPEG with MW of 2000, 5000, 10,000 and 20,000 were coupled to proteins such as dog serum albumin (DA), bovine pancreatic ribonuclease, OA and the constituents of pollen, helminth and bacterial allergens (RAG, Timothy grass pollen, Ascaris suum and Micropolyspora faeni). All these mPEG conjugates depressed markedly the ongoing IgE antibody formation in sensitized animals [mice], in spite of additional injections of the sensitizing dose of the appropriate antigen. The protein allergenicity was either totally abolished or markedly reduced after coupling to mPEG. Conjugates of DA and OA of varying degree of substitution (i.e., number of mPEG molecules attached/protein molecule) were prepared with mPEG of different MW and their immunological properties were assessed. Apparently, for a series of tolerogenic conjugates of the same antigen, there exists some inverse relationship between the substitution degree and the mPEG MW, i.e., a high tolerogenicity level with a concomitant reduction or total loss of allergenicity was achieved with a lower substitution degree utilizing mPEG of increasing MW. Apparently a variety of allergens may be converted with mPEG conjugation to tolerogenic products with a potential for use in the therapy of patients allergic to a wide spectrum of common allergens.

L4 ANSWER 46 OF 48 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1980:283284 Document No.: PREV198070075780; BA70:75780. INHIBITION MURINE REAGINIC ANTIBODY RESPONSES BY NASAL IMMUNO THERAPY WITH **MODIFIED**

**ALLERGEN.** SCHUMACHER M J [Reprint author]; MITCHELL G F. DEP PEDIATR, UNIV ARIZ, HEALTH SCI CENT, TUCSON, ARIZ 85724, USA. International Archives of Allergy and Applied Immunology, (1980) Vol. 62, No. 4, pp. 382-388.
CODEN: IAAAAM. ISSN: 0020-5915. Language: ENGLISH.

AB Optimal conditions were established for induction of reaginic antibodies to Lolium perenne pollen allergens in mice by intranasal dosing of allergens with Bordetella pertussis vaccine. This antibody response could be inhibited by pretreatment of the mice by nasal administration of 100 μg of glutaraldehyde-modified L. perenne allergens 9 times in 3 wk before priming, whereas native allergens, in doses of 5 μg, did not inhibit an Ig[immunoglobulin]E response to subsequent priming. It was not possible to suppress an ongoing reaginic antibody response by intranasal **treatment** with either native allergens, or glutaraldehyde-**modified allergens.** Relevance to immunoprophylaxis of allergic disease is discussed. [L. perenne pollen commonly causes hay fever in humans].

L4 ANSWER 47 OF 48 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

80054590 EMBASE Document No.: 1980054590. Suppression of immunoglobulin E antibodies with **modified allergens.** Sehon A.H.; Lee W.Y.. MRC Canada Group Allergy Res., Dept. Immunol., Fac. Med., Univ. Manitoba, Winnipeg, Canada. Journal of Allergy and Clinical Immunology 64/4 (242-250) 1979.
CODEN: JACIBY. Pub. Country: United States. Language: English.

AB The tolerogenic mPEG allergen conjugates, by virtue of their nonimmunogenicity, appear to satisfy the criteria of a T(s) cell inducer. So far, the nature of the suppressor cells induced by mPEG-modified antigens has not been identified and this problem is under active investigation in our laboratory. As stated earlier, it ought also to be strongly emphasized that many of the tolerogenic PEG conjugates prepared in this laboratory have been shown to be also nonantigenic., i.e., they did not crossreact in vivo or in vitro with IgE and IgG antibodies to the unmodified allergen. Hence, administration of these PEG conjugates into sensitized rats did not induce, by contrast to the unmodified antigen, anaphylactic death of these animals and, therefore, the conjugates appear to have the desirable properties of safe immunotherapeutic agents for the **treatment** of allergic patients. On the basis of all these findings, it may be concluded that a variety of allergenic haptens and allergens may be converted into tolerogenic compounds by conjugation to a variety of nonimmunogenic polymers (e.g., isologous globulins, D-GL, mPEG, PVA) under appropriate conditions and that these conjugates may prove to be safe immunotherapeutic products for the abrogation of a wide spectrum of IGE-mediated **allergies** in man and domestic animals.

L4 ANSWER 48 OF 48 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

77079947 EMBASE Document No.: 1977079947. Chemical modification of crude timothy grass pollen extract. II. Class and specificity of antibodies induced by chemically modified timothy grass pollen extract. Wheeler A.W.; Jenkins P.M.; Moran D.M.. Res. Div., Beecham Pharmaceut., Betchworth, United Kingdom. International Archives of Allergy and Applied Immunology 50/6 (709-728) 1976.
CODEN: IAAAAM. Language: English.

AB The effects have been studied of three different chemical modifications of timothy grass pollen extract on various immunological properties. The ability to induce IgG antibody with specificity for native antigen was least affected by glutaraldehyde **treatment**; IgE antibody production was reduced to a similar extent by all three modifications; there was no increase in IgM production; delayed reactions were reduced. New antigenic determinants were introduced by all the modifications, but the effect was minimal following glutaraldehyde **treatment.** The significance of these results and the potential application of **modified allergen** in hyposensitisation therapy are

discussed.

=> s allergen
L7 117788 ALLERGEN

=> s l7 and modified
L8 2867 L7 AND MODIFIED

=> s l8 and amino acid substitution
L9 18 L8 AND AMINO ACID SUBSTITUTION

=> dup remove l9
PROCESSING COMPLETED FOR L9
L10 8 DUP REMOVE L9 (10 DUPLICATES REMOVED)

=> d l10 1-8 cbib abs

L10 ANSWER 1 OF 8 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN
2003098191 EMBASE Reduction of antigenicity and allergenicity of genetically **modified** egg white **allergen**, ovomucoid third domain.
Mine Y.; Sasaki E.; Zhang J.W.. Y. Mine, Department of Food Science, University of Guelph, Guelph, Ont. N1G2W1, Canada. ymine@uoguelph.ca.
Biochemical and Biophysical Research Communications 302/1 (133-137) 28 Feb 2003.
Refs: 26.
ISSN: 0006-291X. CODEN: BBRCA. Pub. Country: United States. Language: English. Summary Language: English.
AB Ovomucoid (Gal d1) is a major **allergen** in hen egg white, consisting of three tandem domains. In this study, five genetically **modified** third domain (DIII) mutants, which were substituted single or double amino acids within its IgE and IgG epitopes were compared with those prepared and their antigenicity and allergenicity with native analogue using Western immunoblot and enzyme-linked immunosorbent assay. The replacement of phenylalanine at 37 (F37) position with methionine caused drastical loss of IgG and IgE binding activities of human sera derived from egg allergic patients as well as disruption of the α-helix structure which comprises a part of the IgG and IgE epitopes. Substituting glycine at 32 position in conjunction with F37 showed a synergistic effect of decreasing antigenicity. The present study indicated that glycine 32 and phenylalanine 37 have an important role on its antigenicity and allergenicity as well as structural integrity of ovomucoid DIII. .COPYRGT. 2003 Elsevier Science (USA). All rights reserved.

L10 ANSWER 2 OF 8 MEDLINE on STN DUPLICATE 1
2002095800. PubMed ID: 11799381. Linear IgE epitope mapping of the English walnut (Juglans regia) major food **allergen**, Jug r 1. Robotham Jason M; Teuber Suzanne S; Sathe Shridhar K; Roux Kenneth H. (Department of Biological Science and Structural Biology Program, Florida State University, Tallahassee 32306-4370, USA. ) Journal of allergy and clinical immunology, (2002 Jan) 109 (1) 143-9. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.
AB BACKGROUND: Peanut and tree nut allergies can be life-threatening, and they appear to be growing in prevalence. Jug r 1, a 2S albumin seed storage protein, was previously characterized as a major English walnut food **allergen.** OBJECTIVE: We sought to identify the linear IgE-binding epitopes of Jug r 1 and to determine which, if any, amino acids are necessary for this binding to occur. METHODS: Pools of sera from walnut-allergic patients and overlapping peptides synthesized on an activated cellulose membrane were used to screen for IgE-binding epitopes. Mutational analysis of the immunodominant epitope was carried out through single and multisite **amino acid substitutions** . Inhibition assays were performed through use of affinity-purified IgE,

soluble forms of the epitope peptide, and the recombinant 2S albumin, rJug r 1. RESULTS: One immunodominant linear epitope was identified. Amino acid mutations to the epitope demonstrated that the residues RGEE, at positions 36 through 39, were minimally required for IgE binding. Probing of this epitope with sera from each of 20 patients revealed 15 of the sera to be positive. Binding of patients' IgE to the epitope was inhibited with a soluble form of the peptide; however, soluble peptide did not completely inhibit the binding of IgE to the intact rJug r 1. CONCLUSION: One major linear IgE-reactive epitope and its critical core amino acid residues have been identified. Mutation of any of these core amino acids resulted in loss of IgE binding to the epitope, and this points toward the feasibility of reducing allergenicity in genetically **modified** walnuts. However, strong evidence for the existence of conformational epitopes was also obtained.

L10 ANSWER 3 OF 8 MEDLINE on STN
2002084678. PubMed ID: 11811644. The effect of fungal ribosome inactivating proteins upon feeding choice in C. freemani, and indications of a mutualistic relationship with A. restrictus. Environmental mycology. Brandhorst T; Dowd P F; Kenealy W R. (Department of Pediatrics, University of Wisconsin Medical School, University of Wisconsin Hospital and Clinics, Madison 53792, USA.. Tbrandho@facstaff.wisc.edu) . Mycopathologia, (2001) 152 (3) 155-8. Journal code: 7505689. ISSN: 0301-486X. Pub. country: Netherlands. Language: English.

AB Carpophilus freemani beetles' feeding on the fungus Aspergillus nidulans was substantially inhibited when A. nidulans was transformed and induced to secrete the ribosome inactivating protein, restrictocin (genetic source: Aspergillus restrictus). No inhibition of feeding was observed when A. nidulans was transformed and induced to produce an inactive form of restrictocin with a single **amino-acid substitution** in the active site. Similarly, there was no inhibition of feeding upon transgenic strains when the production of restrictocin was not induced. Feeding inhibition of C. freemani by restrictocin requires that the ribonuclease be active and is not due to other characteristics of the protein or the transgenic host fungus.

L10 ANSWER 4 OF 8 MEDLINE on STN DUPLICATE 2
2001209860. PubMed ID: 11298012. How to make foods safer--genetically **modified** foods. Moseley B E. (Reading, Berkshire, UK. ) Allergy, (2001) 56 Suppl 67 61-3. Ref: 7. Journal code: 7804028. ISSN: 0105-4538. Pub. country: Denmark. Language: English.

AB It is the responsibility of companies developing genetically **modified** foods, and of regulatory authorities that approve their marketing, to ensure that they are at least as safe as the traditional foods they are intended to replace in the diet. This requires that any novel material introduced into the food material should not be allergenic. If the novel gene has come from an allergenic source, e.g. nuts, it is necessary to demonstrate using immunological procedures applied to the IgE fractions of pooled sera from individuals with confirmed allergies that the novel protein is non-allergenic. When the novel gene is from a non-allergenic source then it is necessary to demonstrate lack of significant amino acid sequence homology to known **allergens** together with sensitivity to food manufacturing and digestive processes. Consumer confidence in genetically **modified** foods would be significantly improved if hypoallergenic varieties of crops and food products that are currently allergenic could be developed. Techniques such as antisense technology and single site **amino acid substitution** have been shown to have such potential.

L10 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2004 ACS on STN
1998:682138 Document No. 129:301697 Mutants of grass **allergens** not recognized by IgE of allergic patients and their use in specific immunotherapy. Kahlert, Helga; Stuwe, Hans-Thomas; Fiebig, Helmut; Cromwell, Oliver; Becker, Wolf-Meinhard; Bufe, Albrecht; Schramm, Gabriele; Jager, Lothar; Muller, Wolf-Dieter (Merck Patent G.m.b.H.,

Germany). PCT Int. Appl. WO 9843657 A2 19981008, 58 pp. DESIGNATED STATES: W: HU, JP, PL, US; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (German). CODEN: PIXXD2. APPLICATION: WO 1998-EP1507 19980316. PRIORITY: DE 1997-19713001 19970327.

AB Mutants of **allergens** of a grass (Phleum pratense) that stimulate the lymphocyte proliferation and cytokine synthesis in sufferers of pollen allergies, but have significantly lower binding to serum IgE antibodies of patients are described. The **allergens** can be manufactured by expression of the cloned gene for use in immunotherapy of grass allergies. Specifically, the T-cell epitopes of the **allergens** are **modified** and the modification may arise from a spontaneous mutation or by site-specific mutagenesis. T cell epitopes of the Phl p 5 **allergen** were identified and **allergen** derivs. lacking the most significant ones were prepared by site-directed mutagenesis involving **amino acid substitutions** and deletions. The derivs. showed very little **allergen** activity as judged by their inability to inhibit IgE binding to wild-type **allergen.**

L10 ANSWER 6 OF 8 MEDLINE on STN DUPLICATE 3

1998224476. PubMed ID: 9564806. Antagonistic peptides specifically inhibit proliferation, cytokine production, CD40L expression, and help for IgE synthesis by Der p 1-specific human T-cell clones. Fasler S; Aversa G; de Vries J E; Yssel H. (Human Immunology Department, DNAX Research Institute for Molecular and Cellular Biology, Palo Alto, Calif, USA. ) Journal of allergy and clinical immunology, (1998 Apr) 101 (4 Pt 1) 521-30. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Allergic disorders are characterized by IgE antibody responses to a multitude of **allergens** as a result of the ability of these antibodies to specifically bind to high-affinity IgE receptors on mast cells and basophils. This interaction results in receptor activation and release of soluble mediators such as histamine and leukotrienes, which cause allergic reactions in various target organs. Because the synthesis of IgE is tightly regulated by cytokines and CD40 ligand (L) interactions, CD4+ helper T cells are obvious targets, with the aim to modulate **allergen**-induced IgE responses. OBJECTIVES: Because of the central role of **allergen**-specific T-helper type 2 (TH2) cells in the pathway leading to IgE synthesis in vitro and in vivo, we have evaluated the possibility of inhibiting **allergen**-induced activation of these cells by using **allergen**-derived peptides that have been **modified** by single **amino acid substitutions.** METHODS: Three cloned human TH2-like CD4+ T-cell lines, specific for Der p 1, the major **allergen** in house dust, were used in this study. Upon activation with Der p 1 or specific Der p 1-derived wild-type peptides, these T-cell clones produce high levels of IL-4 and IL-5 and low levels of interferon-gamma and IL-2, respectively, and furthermore give help to B cells for the production of IgE in vitro. **Modified** synthetic peptides were generated by the introduction of single **amino acid substitutions** into two different T-cell activation-inducing epitopes on Der p 1. The effects of these **modified** peptides were studied in Der p 1-induced proliferation, cytokine production, and in vitro IgE production assays. RESULTS: Several substituted Der p 1-derived peptides failed to induce T-cell proliferation, in contrast to the native peptides. In addition, some of these peptides acted as antagonists by strongly inhibiting wild-type peptide-induced proliferation as well as the production of interferon-gamma, IL-2, IL-4, and IL-5, although the production of the latter two cytokines was less affected than that of interferon-gamma, even at a 100-fold molar excess of the antagonistic peptides. In addition, the presence of an excess of each of the antagonistic peptides during the activation of Der p I-specific T-cell clones prevented induction of CD40L expression, resulting in a failure of these cells to give help to B cells for the production of IgE in vitro, even in the presence of exogenous IL-4. CONCLUSIONS: Substitution of certain amino acid residues in

immunogenic Der p 1-derived peptides results in the generation of peptides that fail to induce proliferation of Der p 1-specific T-cell clones. In addition, these **modified** peptides have strong antagonistic activities on Der p 1-induced proliferation, cytokine production, and CD40L expression by **allergen**-specific T-cell clones as well as on T cell-mediated IgE production by B cells. These findings suggest that **modified** peptides interfere with **allergen**-induced activation of T cells, including the production of cytokines and the expression of surface molecules important for successful T cell-B cell interactions, and may therefore have therapeutic potential by inhibiting the expansion and function of **allergen**-specific TH2 cells.

L10 ANSWER 7 OF 8 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1998:257635 Document No.: PREV199800257635. Antagonistic peptides specifically inhibit proliferation, cytokine production, CD40L expression, and help for IgE synthesis by Der p 1-specific human T-cell clones. Fasler, Stephan; Aversa, Gregoria; De Vries, Jan E.; Yssel, Hans [Reprint author]. INSERM U454, Hopital Arnaud de Villeneuve, 371 Ave. Doyen Gaston Giraud, 34295 Montpellier Cedex, France. Journal of Allergy and Clinical Immunology, (April, 1998) Vol. 10, No. 4 PART 1, pp. 521-530. print.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

AB Background: Allergic disorders are characterized by IgE antibody responses to a multitude of **allergens** as a result of the ability of these antibodies to specifically bind to high-affinity IgE receptors on mast cells and basophils. This interaction results in receptor activation and release of soluble mediators such as histamine and leukotrienes, which cause allergic reactions in various target organs. Because the synthesis of IgE is tightly regulated by cytokines and CD40 ligand (L) interactions, CD4+ helper T cells are obvious targets, with the aim to modulate **allergen**-induced IgE responses. Objectives: Because of the central role of **allergen**-specific T-helper type 2 (TH2) cells in the pathway leading to IgE synthesis in vitro and in vivo, we have evaluated the possibility of inhibiting **allergen**-induced activation of these cells by using **allergen**-derived peptides that have been **modified** by single **amino acid substitutions.** Methods: Three cloned human TH2-like CD4+ T-cell lines, specific for Der p 1, the major **allergen** in house dust, were used in this study. Upon activation with Der p 1 or specific Der p 1-dervived wild-type peptides, these T-cell clones produce high levels of IL-4 and IL-5 and low levels of interferon-gamma and IL-2, respectively, and furthermore give help to B cells for the production of IgE in vitro. **Modified** synthetic peptides were generated by the introduction of single **amino acid substitutions** into two different T-cell activation-inducing epitopes on Der p 1. The effects of these **modified** peptides were studied in Der p 1-induced proliferation, cytokine production, and in vitro IgE production assays. Results: Several substituted Der p 1-derived peptides failed to induce T-cell proliferation, in contrast to the native peptides. In addition, some of these peptides acted as antagonists by strongly inhibiting wild-type peptide-induced proliferation as well as the production of interferon-gamma, IL-2, IL-4, and IL-5, although the production of the latter two cytokines was less affected than that of interferon-gamma, even at a 100-fold molar excess of the antagonistic peptides. In addition, the presence of an excess of each of the antagonistic peptides during the activation of Der p I-specific T-cell clones prevented induction of CD40L expression, resulting in a failure of these cells to give help to B cells for the production of IgE in vitro, even in the presence of exogenous IL-4. Conclusions: Substitution of certain amino acid residues in immunogenic Der p 1-derived peptides results in the generation of peptides that fail to induce proliferation of Der p 1-specific T-cell clones. In addition, these **modified** peptides have strong antagonistic activities on Der p 1-induced proliferation, cytokine production, and CD40L expression by **allergen**-specific T-cell clones as well as on T cell-mediated IgE production by B cells. These findings suggest that **modified** peptides interfere with **allergen**-induced

activation of T cells, including the production of cytokines and the expression of surface molecules important for successful T cell-B cell interactions, and may therefore have therapeutic potential by inhibiting the expansion and function of **allergen**-specific TH2 cells.

L10 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2004 ACS on STN
1993:579195 Document No. 119:179195 T cell epitopes of the major **allergens** from Dermatophagoides (house dust mite). Garman, Richard D.; Greenstein, Julia L.; Kuo, Mei Chang; Rogers, Bruce L. (Immulogic Pharmaceutical Corp., USA). PCT Int. Appl. WO 9308279 A1 19930429, 176 pp. DESIGNATED STATES: W: AU, CA, FI, HU, JP, KR, NO; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1992-US8637 19921015. PRIORITY: US 1991-777859 19911016; US 1992-881396 19920508.
AB The 4 **allergens** were immunoaffinity-purified from spent mite culture media. Recombinant **allergens** were also prepared by cloning and expressing the cDNA in BL21 cells; amino acid sequence polymorphisms were discovered. T cell epitopic studies and cross reactivity studies are shown. There was no detectable IgE reactivity to any of 56 T cell epitopic peptides screened.

=> s allergen
L11 117788 ALLERGEN

=> s l11 and amino acid substitution
L12 201 L11 AND AMINO ACID SUBSTITUTION

=> s l12 and food
L13 33 L12 AND FOOD

=> dup remove l13
PROCESSING COMPLETED FOR L13
L14 12 DUP REMOVE L13 (21 DUPLICATES REMOVED)

=> d l14 1-12 cbib abs

L14 ANSWER 1 OF 12 MEDLINE on STN DUPLICATE 1
2002211600. PubMed ID: 11944924. Identification and fine mapping of IgG and IgE epitopes in ovomucoid. Mine Yoshinori; Wei Zhang Jie. (Department of Food Science, University of Guelph, Guelph, Ontario, N1G2W1, Canada.. ymine@uoguelph.ca) . Biochemical and biophysical research communications, (2002 Apr 12) 292 (4) 1070-4. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.
AB Ovomucoid is a major **allergen** in hen egg white which causes a serious IgE-mediated **food** allergy reaction. This study determined eight IgG epitopes, 5-11 amino acids in length, and nine IgE epitopes, 5-16 amino acids in length, within the primary sequence in ovomucoid using arrays of overlapping peptides synthesized on cellulose membranes. Pooled sera from eight egg-allergic patients were used to probe the membrane. We also analyzed the amino acids that are critical for antibody binding by substituting a single amino acid within each epitope. Mutational analysis of the epitopes indicated that charged amino acids (aspartic acid, glutamic acid, and lysine) and some hydrophobic (leucine, phenylalanine, and glycine) and polar (serine, threonine, tyrosine, and cystein) amino acids were important for antibody binding. These results provide useful information for the molecular design necessary to reduce the allergenicity of ovomucoid, and a better understanding of structure-function relationships of allergic epitopes in **food** proteins.


L14 ANSWER 2 OF 12 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2002445678 EMBASE Optimal management of atopic dermatitis in infancy.

Moneret-Vautrin D.-A.. D.-A. Moneret-Vautrin, Department of Internal Medicine, Clin. Immunol./Allergol. Univ. Hosp., av. de Lattre de Tassigny, 54035 Nancy, France. Allergie et Immunologie 34/9 (325-329) 2002. Refs: 56.
ISSN: 0397-9148. CODEN: ALGIBW. Pub. Country: France. Language: English. Summary Language: English; French.

AB The necessity to optimise the management of atopic dermatitis of infants needs knowledge of three components: increase of prevalence, extreme frequency of **food** allergy and increase in the frequency of the syndrome of multiple allergies, that frequently develops into asthmatic disease. Management of DA in infancy (first year of life) is based on the global strategy of understanding the physiological Th2 polarisation at birth, that does not allow a re-equilibration of the Th1-Th2 balance that progresses in the first six months of life (in normal infants) making in this period a window of opportunity for sensitisations. Prevention in high-risk children (familial history of atopy) covers the non-exposure to in door polluants (tobacco and volatile orgonic compounds), breast-feeding or a hypoallergenic formula for a hydrolysate of pork and soya proteins or better an extensive hydrolysate of casein. Four situations require moving to an amino acid substitute: failure to thrive, severe atopic dermatitis, a syndrome of multiple **food** allergies, allergy to hydrolysates. Reintroduction of **foods** should be considered with the least delay so as to induce digestive tolerance. It should take into account the clinical development, the intensity of the sensitisation and eventualy depend on a realistic test of introduction. Management of DA searches for recovery of generalized eczema, failure to immediate improvement of quality of life prevention of immediate complications (local sepsis) occeleration of return to **food** tolerance. Prevention of ulterior development of asthma by immedialely introducing measures to diminish respiratory exposure to **allergens** and tobacco is hoped for.

L14 ANSWER 3 OF 12 MEDLINE on STN DUPLICATE 2

2002095800. PubMed ID: 11799381. Linear IgE epitope mapping of the English walnut (Juglans regia) major **food allergen**, Jug r 1.
Robotham Jason M; Teuber Suzanne S; Sathe Shridhar K; Roux Kenneth H. (Department of Biological Science and Structural Biology Program, Florida State University, Tallahassee 32306-4370, USA. ) Journal of allergy and clinical immunology, (2002 Jan) 109 (1) 143-9. Journal code: 1275002.
ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Peanut and tree nut allergies can be life-threatening, and they appear to be growing in prevalence. Jug r 1, a 2S albumin seed storage protein, was previously characterized as a major English walnut **food allergen.** OBJECTIVE: We sought to identify the linear IgE-binding epitopes of Jug r 1 and to determine which, if any, amino acids are necessary for this binding to occur. METHODS: Pools of sera from walnut-allergic patients and overlapping peptides synthesized on an activated cellulose membrane were used to screen for IgE-binding epitopes. Mutational analysis of the immunodominant epitope was carried out through single and multisite **amino acid substitutions.** Inhibition assays were performed through use of affinity-purified IgE, soluble forms of the epitope peptide, and the recombinant 2S albumin, rJug r 1. RESULTS: One immunodominant linear epitope was identified. Amino acid mutations to the epitope demonstrated that the residues RGEE, at positions 36 through 39, were minimally required for IgE binding. Probing of this epitope with sera from each of 20 patients revealed 15 of the sera to be positive. Binding of patients' IgE to the epitope was inhibited with a soluble form of the peptide; however, soluble peptide did not completely inhibit the binding of IgE to the intact rJug r 1. CONCLUSION: One major linear IgE-reactive epitope and its critical core amino acid residues have been identified. Mutation of any of these core amino acids resulted in loss of IgE binding to the epitope, and this points toward the feasibility of reducing allergenicity in genetically modified walnuts. However, strong evidence for the existence of conformational epitopes was also obtained.

L14 ANSWER 4 OF 12 MEDLINE on STN DUPLICATE 3
2002322196. PubMed ID: 12023195. Current understanding of **food allergens**. Lehrer Samuel B; Ayuso Rosalia; Reese Gerald. (Section of Clinical Immunology, Allergy and Rheumatology, Department of Medicine, Tulane University School of Medicine, New Orleans, Louisiana 70112, USA.. sblehrer@tulane.edu) . Annals of the New York Academy of Sciences, (2002 May) 964 69-85. Ref: 39. Journal code: 7506858. ISSN: 0077-8923. Pub. country: United States. Language: English.

AB **Food** allergies are IgE-mediated immunological reactions; this distinguishes them from other adverse reactions to **foods**. Most (>90%) of the recognized **food** allergies are generally thought to be caused by eight **foods** or **food** groups. A number of factors can affect **food** allergy development, including diet and culture, route of exposure, processing, cooking, and digestion. In addition, it is thought that the properties of certain **food** proteins render them more likely to be allergenic than other proteins. Most **food allergens** are major proteins, polyvalent molecules with at least two or more IgE-binding sites, and are recognized as foreign molecules (hence immunogenic). A number of major **food allergens** have been recently characterized, and amino acid sequences determined. Tropomyosin is the only major **allergen** of shrimp. A number of IgE-binding epitopes have been identified in this molecule, though they may vary from one shrimp-allergic individual to another. Single **amino acid substitutions** within epitopes based on that of homologous, nonreactive tropomyosins can substantially enhance or abolish IgE antibody binding. Using the accumulated knowledge of **food allergen** protein structure, the allergenicity of novel proteins to which there has been no prior human exposure has been assessed. This has been based primarily on the lability or resistance of a protein to enzymatic degradation. Clearly, further criteria must be developed to refine this process. In this regard, the development of animal models that have been sufficiently validated as surrogates of human IgE antibody responses is needed for more precise assessment of the allergenic potential of proteins.

L14 ANSWER 5 OF 12 MEDLINE on STN DUPLICATE 4
2002615868. PubMed ID: 12372997. Molecular basis of arthropod cross-reactivity: IgE-binding cross-reactive epitopes of shrimp, house dust mite and cockroach tropomyosins. Ayuso Rosalia; Reese Gerald; Leong-Kee Susan; Plante Matthew; Lehrer Samuel B. (Section of Allergy and Clinical Immunology, Tulane University School of Medicine, 1700 Perdido Street, New Orleans, LA 70112, USA. ) International archives of allergy and immunology, (2002 Sep) 129 (1) 38-48. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Shrimp may cross-react with other crustaceans and mollusks and nonedible arthropods such as insects (cockroach and chironomids), arachnids (house dust mites) and even nematodes. Since the muscle protein tropomyosin has been implicated as a possible cross-reacting **allergen**, this study characterized the IgE-binding epitopes in shrimp tropomyosin, Pen a 1, that cross-react with other allergenic invertebrate tropomyosins in house dust mites (Der p 10, Der f 10) and cockroaches (Per a 7). Pen a 1-reactive sera from shrimp-allergic subjects were used to evaluate the effect on IgE binding of different **amino acid substitutions** in Pen a 1 epitopes based on homologous sequences in Per a 7 and Der p 10/Der f 10. METHODS: Peptides were synthesized spanning the length of Pen a 1 IgE-binding epitopes and **amino acid substitutions** were performed based on homologous amino acid sequences from Per a 7 and Der p 10/Der f 10. RESULTS: 7/8 individually recognized Pen a 1 epitopes (2, 3a, 3b, 4, 5a, 5b and 5c) had an identical amino acid sequence with lobster **allergen**, Hom a 1, 4/8 (3a, 3b, 4 and 5a) with Der p 10 and Der f 10, and 5/8 (2, 3a, 3b, 4 and 5a) with Per a 7. In addition, even homologous regions of other arthropod tropomyosins that differ in one or more amino acids from the sequences of Pen a 1 epitopes are still recognized by shrimp-allergic IgE antibodies. In total, shrimp-allergic

sera recognize 6/8 peptides homologous to Pen a 1 epitopes in Per a 7, 7/8 in Der p 10/Der f 10, and 7/8 epitopes in Hom a 1. CONCLUSIONS: The IgE recognition by shrimp-allergic individuals of identified and/or similar amino acid sequences homologous to Pen a 1 epitopes in mite, cockroach and lobster tropomyosins are the basis of the in vitro cross-reactivity among invertebrate species. Based on amino acid sequence similarity and epitope reactivity, lobster tropomyosin has the strongest and cockroach the least cross-reactivity with shrimp. The clinical relevance of these cross-reactivities in developing allergic reactions to different arthropods needs to be determined.


L14 ANSWER 6 OF 12 EMBASE 
on STN DUPLICATE 5
2001197117 EMBASE Molecular basis of allergic cross-reactivity between group 1 major **allergens** from birch and apple. Holm J.; Baerentzen G.; Gajhede M.; Ipsen H.; Larsen J.N.; Lowenstein H.; Wissenbach M.; Spangfort M.D.. M.D. Spangfort, Biochemical Allergy Research, ALK-Abello A/S, Boge Alle 6-8, DK-2970 Horsholm, Denmark. msp@dk.alk-abello.com. Journal of Chromatography B: Biomedical Sciences and Applications 756/1-2 (307-313) 25 May 2001.
Refs: 19.
ISSN: 0378-4347. CODEN: JCBBEP.
Publisher Ident.: S 0378-4347(01)00089-5. Pub. Country: Netherlands.
Language: English. Summary Language: English.
AB Patients allergic to birch pollen often also react with fruits and vegetables, such as apple. The major cause of cross-reactivity between birch and apple is biochemical and immunological similarity between the major **allergens**, Bet v 1 and Mal d 1, as demonstrated by serological and cellular immunoassays. In addition, birch pollen-specific therapeutic allergy vaccination has been shown to improve allergic symptoms caused by oral ingestion of apple. Detailed analysis of molecular surface areas based on the crystal structure of Bet v 1, and primary sequence alignment, identify potential epitopes for cross-reactive antibodies. Two or more conserved patches are identified when comparing Bet v 1 and Mal d 1, thus providing a molecular model for serological cross-reactivity involving more than one IgE-binding epitope. A minimum of two epitopes would be necessary for cross-linking of receptor bound IgE in functional histamine release assays and skin test. Individual **amino acid substitutions**, as occurring in isoallergenic variation, may, however, have a dramatic effect on epitope integrity if critical residues are affected. Thus, one area large enough to accommodate antibody-binding epitopes shared by all known Mal d 1 isoallergens and variants is identified, as well as areas shared by Bet v 1 and individual Mal d 1 isoallergens or variants. The occurrence of limited epitope coincidence between Bet v 1 and Mal d 1 is in agreement with the observation that some, but not all, birch pollen allergic patients react with apple, and that the epitope repertoire recognised by the IgE of the individual patients determines the degree of cross-reactivity. 

L14 ANSWER 7 OF 12 BIOSIS 
2001:507136 Document No.: PREV200100507136. Effect of **amino acid substitutions** on the IgE binding capacity of major epitopes of the shrimp **allergen** Pen a 1 (tropomyosin): Implications for the development of a hypoallergenic isoform for immunotherapy. Reese, G. [Reprint author]; Ayuso, R. [Reprint author]; Lehrer, S. B. [Reprint author]. Medicine/Clinical Immunology, Tulane University Health Sciences Center, New Orleans, LA, USA. Allergy (Copenhagen), (2001) Vol. 56, No. Supplement 68, pp. 269. print.
Meeting Info.: XXth Congress of the European Academy of Allergology and Clinical Immunology. Berlin, Germany. May 09-13, 2001.
CODEN: LLRGDY. ISSN: 0105-4538. Language: English.

L14 ANSWER 8 OF 12 MEDLINE on STN DUPLICATE 6

2001209860. PubMed ID: 11298012. How to make **foods** safer--genetically modified **foods**. Moseley B E. (Reading, Berkshire, UK. ) Allergy, (2001) 56 Suppl 67 61-3. Ref: 7. Journal code: 7804028. ISSN: 0105-4538. Pub. country: Denmark. Language: English.

AB It is the responsibility of companies developing genetically modified **foods**, and of regulatory authorities that approve their marketing, to ensure that they are at least as safe as the traditional **foods** they are intended to replace in the diet. This requires that any novel material introduced into the **food** material should not be allergenic. If the novel gene has come from an allergenic source, e.g. nuts, it is necessary to demonstrate using immunological procedures applied to the IgE fractions of pooled sera from individuals with confirmed allergies that the novel protein is non-allergenic. When the novel gene is from a non-allergenic source then it is necessary to demonstrate lack of significant amino acid sequence homology to known **allergens** together with sensitivity to **food** manufacturing and digestive processes. Consumer confidence in genetically modified **foods** would be significantly improved if hypoallergenic varieties of crops and **food** products that are currently allergenic could be developed. Techniques such as antisense technology and single site **amino acid substitution** have been shown to have such potential.

L14 ANSWER 9 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN

2001:84448 Document No. 134:99748 The study on characteristics of the gut-associated immune system concerning generation and suppression of **food** allergy. Kaminogawa, Shuichi (Dep. Appl. Biol. Chem., The Univ. Tokyo, Japan). Nippon Nogei Kagaku Kaishi, 75(1), 1-20 (Japanese) 2001. CODEN: NNKKAA. ISSN: 0002-1407. Publisher: Nippon Nogei Kagakkai.

AB A review with 95 refs., on the mol. mechanism of **food** allergy, mechanism of the recognition of **allergens** (αs1-casein and β-lactoglobulin) by T cell and B cell, antigen-specific inhibition of T cell responses to β-lactoglobulin by **amino acid substitution**, induction of TCR antagonism, animal model for **food** allergy, anal. of denaturation of **allergens** by monoclonal antibodies, roles of intestinal intraepithelial T lymphocytes (IEL) and Peyer's patch in gut-associated lymphoid tissue, differentiation and function of IEL, mol. mechanism of oral immune tolerance, application of oral immune tolerance in the treatment of allergy and autoimmune diseases, and suppression of allergic responses by **food** components (lactic acid bacteria, etc.).

L14 ANSWER 10 OF 12 MEDLINE on STN DUPLICATE 7

2000148681. PubMed ID: 10669862. Mutational analysis of the IgE-binding epitopes of P34/Gly m Bd 30K. Helm R M; Cockrell G; Connaughton C; West C M; Herman E; Sampson H A; Bannon G A; Burks A W. (Department of Pediatrics, University of Arkansas for Medical Sciences, Arkansas Children's Nutrition Center, Little Rock, AR 72202, USA. ) Journal of allergy and clinical immunology, (2000 Feb) 105 (2 Pt 1) 378-84. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Peanuts and soybeans are 2 **foods** that have been shown to be responsible for many atopic disorders. Because of their nutritional benefit, soybean proteins are now being used increasingly in a number of **food** products. Previous studies have documented multiple **allergens** in soybean extracts, including glycinin, beta-conglycinin, and the P34/Gly m Bd 30K protein. OBJECTIVE: Our overall goal was to identify soybean-specific **allergens** to begin to understand molecular and immunochemical characteristics of legume proteins. The specific aim of the current investigation was to identify the essential amino acid residues necessary for IgE binding in the 5 distinct immunodominant epitopes of P34/Gly m Bd 30K. METHODS: Serum IgE from 6 clinically sensitive soybean-allergic individuals was used to identify P34/Gly m Bd 30K in the native and single amino acid substituted peptides with use of the SPOTS peptide synthesis technique to determine

critical amino acids required for IgE binding. RESULTS: The intensity of IgE binding and epitope recognition by serum IgE from the individuals varied substantially. With use of serum from 6 clinically soybean-sensitive individuals, 2 of the 5 immunodominant epitopes could be mutagenized to non-IgE binding peptides. CONCLUSIONS: Single-site **amino acid substitution** of the 5 immunodominant epitopes of Gly m Bd 30K with alanine revealed that IgE binding could be reduced or eliminated in epitopes 6 and 16 in the serum obtained from 6 soybean-sensitive patients.

L14 ANSWER 11 OF 12 MEDLINE on STN

2001086248. PubMed ID: 11112857. A soybean G2 glycinin **allergen.** 2. Epitope mapping and three-dimensional modeling. Helm R M; Cockrell G; Connaughton C; Sampson H A; Bannon G A; Beilinson V; Nielsen N C; Burks A W. (Department of Pediatrics, University of Arkansas for Medical Sciences, Arkansas Children's Nutrition Center, Little Rock, AR 72202-3591, USA. ) International archives of allergy and immunology, (2000 Nov) 123 (3) 213-9. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Multiple **allergens** have been documented in soybean extracts. IgE from individuals allergic to soybeans, but not to peanut, has been shown by immunoblot analysis to bind to proteins with a molecular weight of approximately 22 kD. These findings suggested that this unique protein fraction from soybean might be responsible, in part, for soybean allergic reactivity. The objective of the present study was to characterize specific B cell epitopes, to determine if any amino acid was critical to IgE binding and to model the 22-kD G2 soybean **allergen** to the three-dimensional (3-D) phaseolin molecule. METHODS: B cell epitopes were identified using SPOTs peptide analysis. Structural orientation of the IgE-binding regions was mapped to the 3-D phaseolin molecule using molecular modeling of the protein tertiary structure. RESULTS: Eleven linear epitopes, representing 15 amino acid peptide sequences, bound to IgE in the glycinin molecule. These epitopes were predicted to be distributed asymmetrically on the surface of G2 trimers. CONCLUSIONS: Only 1 epitope could be rendered non-IgE binding by alanine substitutions in the peptide. The nonrandom distribution of the IgE binding sites provides new insight into their organization in trimers in 11S complexes of the G2 glycinin **allergen.**



L14 ANSWER 12 OF 12 EMBASE  on STN

1999073286 EMBASE Molecular cloning and epitope analysis of the peanut **allergen** Ara h 3. Rabjohn P.; Helm E.M.; Stanley J.S.; West C.M.; Sampson H.A.; Burks A.W.; Bannon G.A.. G.A. Bannon, Univ. of Arkansas for Med. Sciences, 4301 W. Markham, Little Rock, AR 72205, United States. Bannongarya@exchange.uams.edu. Journal of Clinical Investigation 103/4 (535-542) 15 Feb 1999.

Refs: 39.

ISSN: 0021-9738. CODEN: JCINAO. Pub. Country: United States. Language: English. Summary Language: English.

AB Peanut allergy is a significant IgE-mediated health problem because of the increased prevalence, potential severity, and chronicity of the reaction. Following our characterization of the two peanut **allergens** Ara h 1 and Ara h 2, we have isolated a cDNA clone encoding a third peanut **allergen**, Ara h 3. The deduced amino acid sequence of Ara h 3 shows homology to 11S seed- storage proteins. The recombinant form of this protein was expressed in a bacterial system and was recognized by serum IgE from .apprx.45% of our peanut- allergic patient population. Serum IgE from these patients and overlapping, synthetic peptides were used to map the linear, IgE-binding epitopes of Ara h 3. Four epitopes, between 10 and 15 amino acids in length, were found within the primary sequence, with no obvious sequence motif shared by the peptides. One epitope is recognized by all Ara h 3-allergic patients. Mutational analysis of the epitopes revealed that single amino acid changes within these peptides could lead

to a reduction or loss of IgE binding. By determining which amino acids are critical for IgE binding, it might be possible to alter the Ara h 3 cDNA to encode a protein with a reduced IgE-binding capacity. These results will enable the design of improved diagnostic and therapeutic approaches for **food**-hypersensitivity reactions.

=> s l12 and protein
L15 132 L12 AND PROTEIN

=> s l15 and milks
L16 0 L15 AND MILKS

=> s l15 and milk
L17 3 L15 AND MILK

=> dup remove l17
PROCESSING COMPLETED FOR L17
L18 2 DUP REMOVE L17 (1 DUPLICATE REMOVED)

=> d l18 1-2 cbib abs

L18 ANSWER 1 OF 2 MEDLINE on STN DUPLICATE 1
2003363336. PubMed ID: 12897753. Mutational analysis of major, sequential IgE-binding epitopes in alpha s1-casein, a major cow's **milk allergen.** Cocco Renata R; Jarvinen Kirsi-Marjut; Sampson Hugh A; Beyer Kirsten. (Division of Pediatric Allergy and Immunology and the Jaffe Institute for Food Allergy, Mount Sinai School of Medicine, New York, NY, USA. ) Journal of allergy and clinical immunology, (2003 Aug) 112 (2) 433-7. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Allergy to cow's **milk** is common in early childhood, and no therapy other than avoidance exists. In murine models of peanut allergy, immunotherapy with mutated, engineered, **proteins** appears promising. OBJECTIVE: We sought to identify the critical amino acids (AAs) for immunoglobulin E (IgE) binding within the major B-cell epitopes of alpha(s1)-casein, a major cow's **milk allergen.** This will provide the necessary information to alter the cDNA to encode a **protein** capable of activating **milk** -specific T cells, but with reduced IgE-binding capacity. METHODS: For mutational analysis of the IgE-binding epitopes, peptides of 10-14 AAs in length were synthesized on a derivatized cellulose membrane with single or multiple AA substitutions. Membranes were immunolabeled with pooled sera from 15 cow's-**milk**-allergic patients and with 8 individual sera. RESULTS: With the pooled sera, substitution of a single AA led to complete abrogation of IgE binding to 2 of 8 peptides and diminished binding in the remainder. Substitution of multiple AAs led to an abrogation of binding in the remaining peptides. In 4 of the 8 peptides, the critical AA identified with pooled sera did not result in significant reduction of IgE binding with 1 or more individual patients. For these patients, other critical AAs were identified, indicating a more heterogeneous pattern in IgE recognition. CONCLUSION: This study indicates that single or multiple AA substitutions within IgE-binding epitopes result in reduced binding of **milk**-specific IgE antibodies by patients' sera. However, for future immunotherapeutic interventions with mutated peptides, critical AAs should be evaluated with individual patient sera to determine B-cell-epitope heterogeneity.

L18 ANSWER 2 OF 2 EMBASE 
on STN
2001215007 EMBASE Structure and function of **milk allergens**
. Wal J.M.. Dr. J.M. Wal, Lab. Asso. INRA-CEA d'Immuno-Allerg., Alimentaire, DRM-SPI Bat. 136, 91191 Gif sur Yvette, France. Allergy: European Journal of Allergy and Clinical Immunology, Supplement 56/67 (35-38) 2001.

Refs: 15.
ISSN: 0108-1675. CODEN: ALSUET. Pub. Country: Denmark. Language: English. Summary Language: English.

AB **Proteins** (CMP) involved in **milk** allergy are numerous and heterogeneous, with very few structural or functional common features. This heterogeneity is complicated by their genetic polymorphism, resulting in several variants for each **protein.** These variants are characterized by point substitutions of amino acids or by deletions of peptide fragments of varying size or by post-translational modifications such as phosphorylation or glycosylation. All of these modifications may affect allergenicity. No common molecular structure can be associated with allergenicity, although some homologous regions such as casein phospho-peptides can explain an IgE cross-reactivity. Three-dimensional structure is an important feature in CMP allergenicity but denatured and linear epitopes are also involved. Epitopes are numerous and widely spread along the CMP molecule. They may be located in hydrophobic parts of the molecule where they are inaccessible for IgE antibodies in the native conformation of the **protein** but become bioavailable after digestive processes. Peptides as short as ca. 12-14 amino acid residues may account for a significant part of the allergenicity of the whole molecule, which justifies the need to be careful before proposing any CMP hydrolysate for highly allergenic children.

=> s l12 and egg
L19 5 L12 AND EGG

=> dup remove l19
PROCESSING COMPLETED FOR L19
L20 4 DUP REMOVE L19 (1 DUPLICATE REMOVED)

=> d l20 1-4 cbib abs

L20 ANSWER 1 OF 4 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2004:115020 Document No.: PREV200400105037. Effective reduction of antigenicity of hen **egg** lysozyme by site-specific glycosylation. Usui, Masakatsu; Shimizu, Toshiaki; Goto, Yasuko; Saito, Akira; Kato, Akio [Reprint Author]. Department of Biological Chemistry, Yamaguchi University, Yamaguchi, 753-8515, Japan. kato@agr.yamaguchi-u.ac.jp. FEBS Letters, (16 January 2004) Vol. 557, No. 1-3, pp. 169-173. print. CODEN: FEBLAL. ISSN: 0014-5793. Language: English.

AB Various mutant lysozymes were constructed by genetic modification and secreted in yeast expression system to evaluate the changes in the antigenicity of hen **egg** lysozyme (HEL). Although Arg68, the most critical residue to antigenicity of HEL, was substituted with Gln, the binding of monoclonal antibodies (mAbs) with the mutant lysozyme did not critically reduce, remaining 60% of the binding with mAb. In contrast, glycosylated mutant lysozyme G49N whose glycine was substituted with asparagine dramatically reduced the binding with mAb. The oligomannosyl type of G49N lysozyme reduced binding with mAb to one-fifth, while the polymannosyl type of G49N lysozyme completely diminished the binding with mAb. This suggests that the site-specific glycosylation of lysozyme in the interfacial region of lysozyme-antibody complex is more effective to reduce the antigenicity than the mutation of single **amino acid substitution** in the interfacial region.

L20 ANSWER 2 OF 4 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2003098191 EMBASE Reduction of antigenicity and allergenicity of genetically modified **egg** white **allergen**, ovomucoid third domain.
Mine Y.; Sasaki E.; Zhang J.W.. Y. Mine, Department of Food Science, University of Guelph, Guelph, Ont. N1G2W1, Canada. ymine@uoguelph.ca. Biochemical and Biophysical Research Communications 302/1 (133-137) 28 Feb 2003.

Refs: 26.
ISSN: 0006-291X. CODEN: BBRCA. Pub. Country: United States. Language: English. Summary Language: English.

AB Ovomucoid (Gal d1) is a major **allergen** in hen **egg** white, consisting of three tandem domains. In this study, five genetically modified third domain (DIII) mutants, which were substituted single or double amino acids within its IgE and IgG epitopes were compared with those prepared and their antigenicity and allergenicity with native analogue using Western immunoblot and enzyme-linked immunosorbent assay. The replacement of phenylalanine at 37 (F37) position with methionine caused drastical loss of IgG and IgE binding activities of human sera derived from **egg** allergic patients as well as disruption of the α-helix structure which comprises a part of the IgG and IgE epitopes. Substituting glycine at 32 position in conjunction with F37 showed a synergistic effect of decreasing antigenicity. The present study indicated that glycine 32 and phenylalanine 37 have an important role on its antigenicity and allergenicity as well as structural integrity of ovomucoid DIII. 

L20 ANSWER 3 OF 4 MEDLINE on STN DUPLICATE 1

2002211600. PubMed ID: 11944924. Identification and fine mapping of IgG and IgE epitopes in ovomucoid. Mine Yoshinori; Wei Zhang Jie. (Department of Food Science, University of Guelph, Guelph, Ontario, N1G2W1, Canada.. ymine@uoguelph.ca) . Biochemical and biophysical research communications, (2002 Apr 12) 292 (4) 1070-4. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB Ovomucoid is a major **allergen** in hen **egg** white which causes a serious IgE-mediated food allergy reaction. This study determined eight IgG epitopes, 5-11 amino acids in length, and nine IgE epitopes, 5-16 amino acids in length, within the primary sequence in ovomucoid using arrays of overlapping peptides synthesized on cellulose membranes. Pooled sera from eight **egg**-allergic patients were used to probe the membrane. We also analyzed the amino acids that are critical for antibody binding by substituting a single amino acid within each epitope. Mutational analysis of the epitopes indicated that charged amino acids (aspartic acid, glutamic acid, and lysine) and some hydrophobic (leucine, phenylalanine, and glycine) and polar (serine, threonine, tyrosine, and cystein) amino acids were important for antibody binding. These results provide useful information for the molecular design necessary to reduce the allergenicity of ovomucoid, and a better understanding of structure-function relationships of allergic epitopes in food proteins.


L20 ANSWER 4 OF 4 MEDLINE on STN

1999254832. PubMed ID: 10323253. Association of polymorphisms in the beta2-adrenoreceptor gene with higher levels of parasitic infection. Ramsay C E; Hayden C M; Tiller K J; Burton P R; Hagel I; Palenque M; Lynch N R; Goldblatt J; LeSouef P N. (Department of Paediatrics, University of Western Australia, Perth, Australia. ) Human genetics, (1999 Mar) 104 (3) 269-74. Journal code: 7613873. ISSN: 0340-6717. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB The diminishing incidence of parasitic infection in westernised societies has been suggested to result in an increased prevalance of asthma. Asthma is a polygenic disease and genome screens have shown that genes on chromosome 5q31-33 are strongly linked to the disease. The gene for the beta2-adrenoreceptor is located in this region and two polymorphisms have been identified that result in amino acid changes at positions 16 (ArgGly) and 27 (GlnGlu). To determine whether these polymorphisms influence asthma and parasitic infection, a genotype/phenotype study has been performed on a cohort of 126 children from Coche Island in Venezuela. There is a high incidence of asthma on the island and intestinal helminthiasis is endemic. Genotyping for both polymorphisms was carried out by using the polymerase chain reaction and allele-specific

oligonucleotide hybridisation. Genotype frequencies in this cohort were consistent with other studies and both polymorphisms were in significant linkage disequilibrium. Individuals who were homozygous for Arg16 had significantly higher levels of specific IgE to Ascaris lumbricoides (P=0.002), significantly higher A. lumbricoides **egg** counts (P<0.001) and significantly larger wheal sizes following skin-prick testing with A. lumbricoides **allergen** (P=0.008). There was no association between either polymorphism and total serum IgE or asthma in this population. A combination of mast cell degranulation and the lung migratory phase of A. lumbricoides larvae may result in bronchoconstriction in infected individuals. These results suggest that the Gly 16 allele confers resistance to high levels of parasitic infection in this population. An alternative explanation for the association is that it may be the result of linkage disequilibrium with other genes in the chromosome 5q31-33 region.

=> s l12 and fish
L21 0 L12 AND FISH

=> s l12 and crustaceans
L22 5 L12 AND CRUSTACEANS

=> dup remove l22
PROCESSING COMPLETED FOR L22
L23 1 DUP REMOVE L22 (4 DUPLICATES REMOVED)

=> d l23 cbib abs

L23 ANSWER 1 OF 1 MEDLINE on STN DUPLICATE 1
2002615868. PubMed ID: 12372997. Molecular basis of arthropod cross-reactivity: IgE-binding cross-reactive epitopes of shrimp, house dust mite and cockroach tropomyosins. Ayuso Rosalia; Reese Gerald; Leong-Kee Susan; Plante Matthew; Lehrer Samuel B. (Section of Allergy and Clinical Immunology, Tulane University School of Medicine, 1700 Perdido Street, New Orleans, LA 70112, USA. ) International archives of allergy and immunology, (2002 Sep) 129 (1) 38-48. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Shrimp may cross-react with other **crustaceans** and mollusks and nonedible arthropods such as insects (cockroach and chironomids), arachnids (house dust mites) and even nematodes. Since the muscle protein tropomyosin has been implicated as a possible cross-reacting **allergen**, this study characterized the IgE-binding epitopes in shrimp tropomyosin, Pen a 1, that cross-react with other allergenic invertebrate tropomyosins in house dust mites (Der p 10, Der f 10) and cockroaches (Per a 7). Pen a 1-reactive sera from shrimp-allergic subjects were used to evaluate the effect on IgE binding of different **amino acid substitutions** in Pen a 1 epitopes based on homologous sequences in Per a 7 and Der p 10/Der f 10. METHODS: Peptides were synthesized spanning the length of Pen a 1 IgE-binding epitopes and **amino acid substitutions** were performed based on homologous amino acid sequences from Per a 7 and Der p 10/Der f 10. RESULTS: 7/8 individually recognized Pen a 1 epitopes (2, 3a, 3b, 4, 5a, 5b and 5c) had an identical amino acid sequence with lobster **allergen**, Hom a 1, 4/8 (3a, 3b, 4 and 5a) with Der p 10 and Der f 10, and 5/8 (2, 3a, 3b, 4 and 5a) with Per a 7. In addition, even homologous regions of other arthropod tropomyosins that differ in one or more amino acids from the sequences of Pen a 1 epitopes are still recognized by shrimp-allergic IgE antibodies. In total, shrimp-allergic sera recognize 6/8 peptides homologous to Pen a 1 epitopes in Per a 7, 7/8 in Der p 10/Der f 10, and 7/8 epitopes in Hom a 1. CONCLUSIONS: The IgE recognition by shrimp-allergic individuals of identified and/or similar amino acid sequences homologous to Pen a 1 epitopes in mite, cockroach and lobster tropomyosins are the basis of the in vitro cross-reactivity among invertebrate species. Based on amino acid

sequence similarity and epitope reactivity, lobster tropomyosin has the strongest and cockroach the least cross-reactivity with shrimp. The clinical relevance of these cross-reactivities in developing allergic reactions to different arthropods needs to be determined.


=> s l12 and mollusks
L24 5 L12 AND MOLLUSKS

=> dup remove l24
PROCESSING COMPLETED FOR L24
L25 1 DUP REMOVE L24 (4 DUPLICATES REMOVED)

=> d l25 cbib abs

L25 ANSWER 1 OF 1 MEDLINE on STN DUPLICATE 1
2002615868. PubMed ID: 12372997. Molecular basis of arthropod cross-reactivity: IgE-binding cross-reactive epitopes of shrimp, house dust mite and cockroach tropomyosins. Ayuso Rosalia; Reese Gerald; Leong-Kee Susan; Plante Matthew; Lehrer Samuel B. (Section of Allergy and Clinical Immunology, Tulane University School of Medicine, 1700 Perdido Street, New Orleans, LA 70112, USA. ) International archives of allergy and immunology, (2002 Sep) 129 (1) 38-48. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.
AB BACKGROUND: Shrimp may cross-react with other crustaceans and **mollusks** and nonedible arthropods such as insects (cockroach and chironomids), arachnids (house dust mites) and even nematodes. Since the muscle protein tropomyosin has been implicated as a possible cross-reacting **allergen**, this study characterized the IgE-binding epitopes in shrimp tropomyosin, Pen a 1, that cross-react with other allergenic invertebrate tropomyosins in house dust mites (Der p 10, Der f 10) and cockroaches (Per a 7). Pen a 1-reactive sera from shrimp-allergic subjects were used to evaluate the effect on IgE binding of different **amino acid substitutions** in Pen a 1 epitopes based on homologous sequences in Per a 7 and Der p 10/Der f 10. METHODS: Peptides were synthesized spanning the length of Pen a 1 IgE-binding epitopes and **amino acid substitutions** were performed based on homologous amino acid sequences from Per a 7 and Der p 10/Der f 10. RESULTS: 7/8 individually recognized Pen a 1 epitopes (2, 3a, 3b, 4, 5a, 5b and 5c) had an identical amino acid sequence with lobster **allergen**, Hom a 1, 4/8 (3a, 3b, 4 and 5a) with Der p 10 and Der f 10, and 5/8 (2, 3a, 3b, 4 and 5a) with Per a 7. In addition, even homologous regions of other arthropod tropomyosins that differ in one or more amino acids from the sequences of Pen a 1 epitopes are still recognized by shrimp-allergic IgE antibodies. In total, shrimp-allergic sera recognize 6/8 peptides homologous to Pen a 1 epitopes in Per a 7, 7/8 in Der p 10/Der f 10, and 7/8 epitopes in Hom a 1. CONCLUSIONS: The IgE recognition by shrimp-allergic individuals of identified and/or similar amino acid sequences homologous to Pen a 1 epitopes in mite, cockroach and lobster tropomyosins are the basis of the in vitro cross-reactivity among invertebrate species. Based on amino acid sequence similarity and epitope reactivity, lobster tropomyosin has the strongest and cockroach the least cross-reactivity with shrimp. The clinical relevance of these cross-reactivities in developing allergic reactions to different arthropods needs to be determined.


=> s l12 and insects
L26 6 L12 AND INSECTS

=> dup remove l26
PROCESSING COMPLETED FOR L26
L27 2 DUP REMOVE L26 (4 DUPLICATES REMOVED)

=> d l27 1-2 cbib abs

L27 ANSWER 1 OF 2 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2002:182414 Document No.: PREV200200182414. Cockroach **allergen** Bla g 2: Structure, function, and implications for allergic sensitization. Pomes, Anna [Reprint author]; Chapman, Martin D.; Vailes, Lisa D.; Blundell, Tom L.; Dhanaraj, Venugopal. INDOOR Biotechnologies, Inc., 1216 Harris Street, Charlottesville, VA, 22903, USA. apomes@inbio.com. American Journal of Respiratory and Critical Care Medicine, (February 1, 2002) Vol. 165, No. 3, pp. 391-397. print.
ISSN: 1073-449X. Language: English.

AB Exposure to German cockroach (Blatella germanica) **allergens** is associated with the development of chronic respiratory diseases, especially asthma. The mechanism by which allergic patients develop specific immunoglobulin E (IgE) responses to environmental **allergens** is unknown. However, recent reports provided evidence that enzyme activity, especially proteolytic activity, was a major contributor to allergenicity. Bla g 2 is one of the most potent cockroach **allergens** (prevalence of IgE responses of 60 to 80%) and shows homology to the aspartic proteinase family of enzymes. We investigated whether the allergenicity of Bla g 2 was linked to its putative enzymatic function. A molecular model of Bla g 2, based on the high resolution crystal structures of pepsin and chymosin, showed that the overall three-dimensional structure of Bla g 2 was similar to that of aspartic proteinases with a well-defined binding pocket. However, critical **amino acid substitutions** in the catalytic triads and in the "flap" region of the molecule suggested that Bla g 2 was inactive and homologous to mammalian pregnancy-associated glycoproteins. This was confirmed experimentally by enzyme assay. The results show dissociation between enzymatic activity and allergenicity for Bla g 2 and suggest that other genetic and environmental factors are important determinants of sensitization.

L27 ANSWER 2 OF 2 MEDLINE on STN DUPLICATE 1
2002615868. PubMed ID: 12372997. Molecular basis of arthropod cross-reactivity: IgE-binding cross-reactive epitopes of shrimp, house dust mite and cockroach tropomyosins. Ayuso Rosalia; Reese Gerald; Leong-Kee Susan; Plante Matthew; Lehrer Samuel B. (Section of Allergy and Clinical Immunology, Tulane University School of Medicine, 1700 Perdido Street, New Orleans, LA 70112, USA. ) International archives of allergy and immunology, (2002 Sep) 129 (1) 38-48. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Shrimp may cross-react with other crustaceans and mollusks and nonedible arthropods such as **insects** (cockroach and chironomids), arachnids (house dust mites) and even nematodes. Since the muscle protein tropomyosin has been implicated as a possible cross-reacting **allergen**, this study characterized the IgE-binding epitopes in shrimp tropomyosin, Pen a 1, that cross-react with other allergenic invertebrate tropomyosins in house dust mites (Der p 10, Der f 10) and cockroaches (Per a 7). Pen a 1-reactive sera from shrimp-allergic subjects were used to evaluate the effect on IgE binding of different **amino acid substitutions** in Pen a 1 epitopes based on homologous sequences in Per a 7 and Der p 10/Der f 10. METHODS: Peptides were synthesized spanning the length of Pen a 1 IgE-binding epitopes and **amino acid substitutions** were performed based on homologous amino acid sequences from Per a 7 and Der p 10/Der f 10. RESULTS: 7/8 individually recognized Pen a 1 epitopes (2, 3a, 3b, 4, 5a, 5b and 5c) had an identical amino acid sequence with lobster **allergen**, Hom a 1, 4/8 (3a, 3b, 4 and 5a) with Der p 10 and Der f 10, and 5/8 (2, 3a, 3b, 4 and 5a) with Per a 7. In addition, even homologous regions of other arthropod tropomyosins that differ in one or more amino acids from the sequences of Pen a 1 epitopes are still recognized by shrimp-allergic IgE antibodies. In total, shrimp-allergic sera recognize 6/8 peptides homologous to Pen a

1 epitopes in Per a 7, 7/8 in Der p 10/Der f 10, and 7/8 epitopes in Hom a 1. CONCLUSIONS: The IgE recognition by shrimp-allergic individuals of identified and/or similar amino acid sequences homologous to Pen a 1 epitopes in mite, cockroach and lobster tropomyosins are the basis of the in vitro cross-reactivity among invertebrate species. Based on amino acid sequence similarity and epitope reactivity, lobster tropomyosin has the strongest and cockroach the least cross-reactivity with shrimp. The clinical relevance of these cross-reactivities in developing allergic reactions to different arthropods needs to be determined.


=> s l12 and molds
L28 0 L12 AND MOLDS

=> s l12 and mold
L29 5 L12 AND MOLD

=> dup remove l29
PROCESSING COMPLETED FOR L29
L30 1 DUP REMOVE L29 (4 DUPLICATES REMOVED)

=> d l30 cbib abs

L30 ANSWER 1 OF 1 MEDLINE on STN DUPLICATE 1
96381534. PubMed ID: 8789547. An allergenic polypeptide representing a variable region of hsp 70 cloned from a cDNA library of Cladosporium herbarum. Zhang L; Muradia G; De Vouge M W; Rode H; Vijay H M. (Bureau of Drug Research, Health Canada, Ottawa. ) Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (1996 Jan) 26 (1) 88-95. Journal code: 8906443. ISSN: 0954-7894. Pub. country: ENGLAND: United Kingdom. Language: English.

AB BACKGROUND: Extracts of Cladosporium herbarum, a major source of fungal aeroallergens, exhibit a complex profile of IgE-binding proteins. Yields of conventionally purified **allergens** from this **mold** have been insufficient to permit further molecular analyses. OBJECTIVE: To enhance and simplify the purification of **allergens** from C. herbarum, we have sought to use recombinant DNA techniques to clone, identify and bacterially express immunoselected C. herbarum **allergens.** METHODS: We constructed a cDNA library in lambda ZAP II using mRNA isolated from C. herbarum. From this library, phage clones encoding a new **allergen** were immunoselected using pooled human atopic IgE. The cloned cDNA was excised from the phage vector as a recombinant pBluescript II SK-phagemid and sequenced. Expression of the recombinant **allergen** was carried out in E. coli XL1-blue transformants of the phagemid. Bacterial lysates from cells induced to express the cloned **allergen** were immunoblotted and probed with individual human atopic IgEs. RESULTS: The cDNA clone encodes a 278 amino acid polypeptide homologous to the C-terminal portion of 70 kDa heat shock protein (hsp 70). The polypeptide possesses features common to other hsps 70, i.e. a similar hydropathic profile and a variable C-terminal region with conserved sequence at the very C-terminus. Binding of the recombinant peptide to IgE from 38% of atopic sera or plasma from individuals allergic to C. herbarum was demonstrated. CONCLUSION: These results indicate that **amino acid substitutions** are relatively conserved even in the variable C-terminal regions of hsp 70 species. Thus, this study should draw attention to the possibility of induction of anaphylactic responses in a sensitized individual when hsp 70 from any pathogenic species is administered for vaccination.

=> s l12 and dust
L31 46 L12 AND DUST

=> dup remove l31

PROCESSING COMPLETED FOR L31
L32 26 DUP REMOVE L31 (20 DUPLICATES REMOVED)

=> d l32 1-26 cbib abs

L32 ANSWER 1 OF 26 MEDLINE on STN
2003253972. PubMed ID: 12778490. The immunodominant epitope of lipocalin **allergen** Bos d 2 is suboptimal for human T cells. Kinnunen Tuure; Buhot Cecile; Narvanen Ale; Rytkonen-Nissinen Marja; Saarelainen Soili; Pouvelle-Moratille Sandra; Rautiainen Jaakko; Taivainen Antti; Maillere Bernard; Mantyjarvi Rauno; Virtanen Tuomas. (Department of Clinical Microbiology, University of Kuopio, Finland. ) European journal of immunology, (2003 Jun) 33 (6) 1717-26. Journal code: 1273201. ISSN: 0014-2980. Pub. country: Germany: Germany, Federal Republic of. Language: English.
AB We have proposed earlier that the poor capacity of the lipocalin **allergen** Bos d 2 to stimulate highly allergic subjects' peripheral blood mononuclear cells could be ascribed to endogenous lipocalins and could be related to the allergenic potential of the molecule. Here, we have characterized the proliferative and cytokine responses of human T cell clones against the immunodominant epitope of Bos d 2. We observed, for clone F1-9, that a substitution of aspartic acid for asparagine in the core region of the epitope increased the stimulatory capacity of the peptide about 100-fold in comparison with the natural peptide. For clone K3-2, from a different patient, the substitution of lysine for glutamine or isoleucine for leucine in the core region resulted in about 30-fold and 10-fold increases in the stimulatory capacity of the peptides, respectively. The clones also recognized self-protein-derived peptides but not the peptides derived from other lipocalins. We suggest that the poor recognition of the immunodominant epitope of Bos d 2 can be a factor accounting for Bos d 2-allergic subjects' weak cellular responses. Suboptimal recognition of self and **allergen** epitopes by T cells may be of significance for the allergenicity of proteins.

L32 ANSWER 2 OF 26 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2002303262 EMBASE The Dermatophagoides pteronyssinus group 2 **allergen** contains a universally immunogenic T cell epitope. Wu B.; Vander Elst L.; Carlier V.; Jacquemin M.G.; Saint-Remy J.-M.R.. Dr. J.-M.R. Saint-Remy, Ctr. for Molec. and Vascular Biology, University of Leuven, Campus Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium. jeanmarie.saint-remy@med.kuleuven.ac.be. Journal of Immunology 169/5 (2430-2435) 1 Sep 2002.
Refs: 34.
ISSN: 0022-1767. CODEN: JOIMA3. Pub. Country: United States. Language: English. Summary Language: English.
AB The use of T cell epitope-containing peptides for the induction of anergy in **allergen** sensitization is limited by genetic restriction that could be circumvented by using universally immunogenic epitopes. We attempted to identify such epitopes on Dermatophagoidespteronyssinus group 2 **allergen** (Der p 2), a major **allergen** of D. pteronyssinus T cells from BALB/c (H-2(d)), C57BL/6 (H-2(b)), C3H (H-2(k)), and SJL (H-2(s)) mice that were immunized with rDer p 2, recognized an immunodominant region encompassing residues 21-35. A synthetic 21-35 peptide (p21-35) induced strong dose-dependent in vitro T cell proliferation with cells of the four mouse strains and required processing for MHC class II presentation. Substitution of Ile(28) with Ala resulted in reduction of T cell proliferation in each strain. Ile(28) could represent an important MHC class II anchoring residue for T cell response to p21-35. An immunodominant T cell epitope of Der p 2 therefore behaves as a universal epitope and could be a suitable candidate for T cell anergy induction.

L32 ANSWER 3 OF 26 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2002:566481 The Genuine Article (R) Number: 568NL. Stimulatory and inhibitory

epitopes in the T cell responses of mice to Der p 1. Jarnicki A G; Thomas W R (Reprint). Univ Western Australia, Ctr Child Hlth Res, TVW Telethon Inst Child Hlth Res, POB 100, Perth, WA 6872, Australia (Reprint); Univ Western Australia, Ctr Child Hlth Res, TVW Telethon Inst Child Hlth Res, Perth, WA 6872, Australia. CLINICAL AND EXPERIMENTAL ALLERGY (JUN 2002) Vol. 32, No. 6, pp. 942-950. Publisher: BLACKWELL PUBLISHING LTD. P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND. ISSN: 0954-7894. Pub. country: Australia. Language: English.

*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Background The responses of mice to the mite **allergen** Der p 1 have been used to study the mechanisms of allergic sensitization and the development of new types of immunotherapy. Many of the studies require a knowledge of the T cell epitopes, and because Der p 1 is polymorphic, the effect of natural **amino acid substitution** in the **allergen**, The intranasal administration of peptides containing T cell epitopes can induce a mucosal tolerance but it is not known if the major activity is limited to stimulatory peptides and if, as found for autoimmunity, some epitopes are not inhibitory.

Objective To determine and compare the sequences of Der p 1 which contain stimulatory epitopes for the high responding H-2(b) and H-2(q) mice and the sequences which induce tolerance by intranasal administration of peptides.

Methods T cell responses of mice immunized with Der p 1 were measured by in vitro T Cell stimulation assays so an extensive study of epitope recognition and intranasal tolerance could be made. Synthetic peptides were used to examine the stimulatory and inhibitory ability of all Der p 1 sequences and to map the major H-2(b) epitope in detail. This included the effect of the common polymorphic amino acid 124 substitution found within this epitope.

Results Three and two regions. respectively, were found to contain stimulatory T cell epitopes for H-2(b) and H-2(q) mice. The peptides in these regions were also the most active at inducing intranasal tolerance for the responding haplotype. The correspondence between inhibitory and stimulatory peptides was maintained for the fine mapping of the major H-2(b) epitope. This was found about a core region of 118-126 which was overlapping but separate to a consensus sequence for the binding of endogeneous peptides. Peptides with alanine at the naturally polymorphic residue 124 stimulated and inhibited responses to Der p 1 more effectively. while peptides with the valine 124 variant were immunogenic but poorly cross-reactive.

Conclusions The intranasal administration of peptides representing each of five epitopes recognized by two strains of mice were able to induce mucosal tolerance and the major tolerizing activity was limited to these epitopes. The position of the core major epitope for C57 mice, which differs from a previously predicted epitope. and its specificity for the natural alanine 124 variant is described.

L32 ANSWER 4 OF 26 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

2002:118980 The Genuine Article (R) Number: 517PA. Cockroach **allergen** Bla g 2 - Structure, function, and implications for allergic sensitization . Pomes A (Reprint); Chapman M D; Vailes L D; Blundell T L; Dhanaraj V. INDOOR Biotechnol Inc, 1216 Harris St, Charlottesville, VA 22903 USA (Reprint); Univ Virginia, Dept Internal Med, Asthma & Allerg Dis Ctr, Charlottesville, VA USA; Univ Cambridge, Dept Biochem, Cambridge CB2 1QW, England. AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE (1 FEB 2002) Vol. 165, No. 3, pp. 391-397. Publisher: AMER THORACIC SOC. 1740 BROADWAY, NEW YORK, NY 10019-4374 USA. ISSN: 1073-449X. Pub. country: USA; England. Language: English.

*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Exposure to German cockroach (Blattella germanica) **allergens** is associated with the development of chronic respiratory diseases, especially asthma. The mechanism by which allergic patients develop specific immunoglobulin E (IgE) responses to environmental **allergens** is unknown. However, recent reports provided evidence that enzyme activity, especially proteolytic activity, was a major

contributor to allergenicity. Bla g 2 is one of the most potent cockroach **allergens** (prevalence of IgE responses of 60 to 80%) and shows homology to the aspartic proteinase family of enzymes. We investigated whether the allergenicity of Bla g 2 was linked to its putative enzymatic function. A molecular model of Bla g 2, based on the high resolution crystal structures of pepsin and chymosin, showed that the overall three-dimensional structure of Bla g 2 was similar to that of aspartic proteinases with a well-defined binding pocket. However, critical **amino acid substitutions** in the catalytic triads and in the "flap" region of the molecule suggested that Bla g 2 was inactive and homologous to mammalian pregnancy-associated glycoproteins. This was confirmed experimentally by enzyme assay. The results show dissociation between enzymatic activity and allergenicity for Bla g 2 and suggest that other genetic and environmental factors are important determinants of sensitization.

L32 ANSWER 5 OF 26 MEDLINE on STN DUPLICATE 1

2002615868. PubMed ID: 12372997. Molecular basis of arthropod cross-reactivity: IgE-binding cross-reactive epitopes of shrimp, house **dust** mite and cockroach tropomyosins. Ayuso Rosalia; Reese Gerald; Leong-Kee Susan; Plante Matthew; Lehrer Samuel B. (Section of Allergy and Clinical Immunology, Tulane University School of Medicine, 1700 Perdido Street, New Orleans, LA 70112, USA. ) International archives of allergy and immunology, (2002 Sep) 129 (1) 38-48. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Shrimp may cross-react with other crustaceans and mollusks and nonedible arthropods such as insects (cockroach and chironomids), arachnids (house **dust** mites) and even nematodes. Since the muscle protein tropomyosin has been implicated as a possible cross-reacting **allergen**, this study characterized the IgE-binding epitopes in shrimp tropomyosin, Pen a 1, that cross-react with other allergenic invertebrate tropomyosins in house **dust** mites (Der p 10, Der f 10) and cockroaches (Per a 7). Pen a 1-reactive sera from shrimp-allergic subjects were used to evaluate the effect on IgE binding of different **amino acid substitutions** in Pen a 1 epitopes based on homologous sequences in Per a 7 and Der p 10/Der f 10. METHODS: Peptides were synthesized spanning the length of Pen a 1 IgE-binding epitopes and **amino acid substitutions** were performed based on homologous amino acid sequences from Per a 7 and Der p 10/Der f 10. RESULTS: 7/8 individually recognized Pen a 1 epitopes (2, 3a, 3b, 4, 5a, 5b and 5c) had an identical amino acid sequence with lobster **allergen**, Hom a 1, 4/8 (3a, 3b, 4 and 5a) with Der p 10 and Der f 10, and 5/8 (2, 3a, 3b, 4 and 5a) with Per a 7. In addition, even homologous regions of other arthropod tropomyosins that differ in one or more amino acids from the sequences of Pen a 1 epitopes are still recognized by shrimp-allergic IgE antibodies. In total, shrimp-allergic sera recognize 6/8 peptides homologous to Pen a 1 epitopes in Per a 7, 7/8 in Der p 10/Der f 10, and 7/8 epitopes in Hom a 1. CONCLUSIONS: The IgE recognition by shrimp-allergic individuals of identified and/or similar amino acid sequences homologous to Pen a 1 epitopes in mite, cockroach and lobster tropomyosins are the basis of the in vitro cross-reactivity among invertebrate species. Based on amino acid sequence similarity and epitope reactivity, lobster tropomyosin has the strongest and cockroach the least cross-reactivity with shrimp. The clinical relevance of these cross-reactivities in developing allergic reactions to different arthropods needs to be determined.


L32 ANSWER 6 OF 26 EMBASE  on STN

2002065685 EMBASE Monoclonal antibodies to recombinant Der f 2 and development of a two-site ELISA sensitive to major Der f 2 isoallergen in Korea. Jeong K.Y.; Jin H.S.; Oh S.H.; Hong C.-S.; Lee I.-Y.; Ree H.-I.; Yong T.-S.. T.-S. Yong, Institute of Tropical Medicine, Brain Korea 21 Proj. for Med. Coll., Yonsei University, Seoul 120-752, Korea, Republic

of. Allergy: European Journal of Allergy and Clinical Immunology 57/1 (29-34) 2002.
Refs: 24.
ISSN: 0105-4538. CODEN: LLRGDY. Pub. Country: Denmark. Language: English. Summary Language: English.

AB Background: Der f 2 is a major sensitizing **allergen** in patients allergic to house **dust** mites worldwide. Isoforms of Der f 2 have been reported and are known to have different antigenicities. The aim of this study was to facilitate antigenic analysis and to develop an improved method for the detection of Der f 2 isoallergen, which is prevalent in Korea. Methods: A two-site ELISA was developed with monoclonal antibodies (mAbs) which were produced against recombinant Der f 2 (rDer f 2) and applied to assess Der f 2 in bedding samples. Results: A major isoform of Der f 2, found in Korea, was found to have amino acid variations especially at position 100 from lysine to glutamic acid, which is known to reduce significantly the binding affinity of mAbs when used to assess group 2 **allergens.** The detection limit of the developed two-site ELISA was determined to be about 8 ng/ml with rDer f 2 and 1 μg/ml with Dermatophagoides farinae crude extract. The average amount of Der f 2 in **dust** obtained from bedding samples from 89 homes in Seoul was estimated to be 25.61 ± 10.70 μg/g **dust.** Conclusions: Assays using mAbs for rDer f 2 could be useful for the assessment of environmental **allergen** exposure and mAbs could be used to further characterize the isoallergens of Der f 2.

L32 ANSWER 7 OF 26 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2001206312 EMBASE **Allergens** of wild house **dust** mites: Environmental Der p 1 and Der p 2 sequence polymorphisms. Smith W.-A.; Hales B.J.; Jarnicki A.G.; Thomas W.R.. Dr. W.R. Thomas, TVW Telethon Inst. Child Hlth. Res., PO Box 855, West Perth, WA 6872, Australia. Journal of Allergy and Clinical Immunology 107/6 (985-992) 2001.
Refs: 25.
ISSN: 0091-6749. CODEN: JACIBY. Pub. Country: United States. Language: English. Summary Language: English.

AB Background: Sequence diversity is a common feature of mite **allergens.** Previous studies, using predominantly commercial mite clones, have described several polymorphic residues for Der p 1 and Der p 2. Objective: This study aimed at determining the occurrence of sequence diversity in environmental mite isolates. Methods: Mites were isolated from houses in Perth and Sydney, Australia. Total RNA was extracted from 1 to 30 Perth mites, and cDNA was synthesized by reverse transcriptase PCR. Der p 1 and Der p 2 cDNAs were PCR amplified and sequenced. Genomic Der p 1 DNA was amplified from whole Sydney mites directly by PCR and then sequenced. Results: Twelve Der p 1 and 9 Der p 2 cDNA clones and 3 Der p 1 genomic DNA were analyzed and showed a high frequency of amino acid polymorphisms. Der p 2 displayed a clear pattern of divergence toward 2 alleles that differed by 4 amino acids and had characteristic silent nucleotide changes. The pattern for Der p 1 was different and unusual, with almost no silent nucleotide substitutions but frequent sporadic missense changes. Proliferative responses of peripheral blood mononuclear cells to peptides containing polymorphic residues of Der p 1 were detected in 8 of 19 subjects, with stimulation being found only for either one of the variant forms of the peptides. However, the responses to variants of whole recombinant **allergens** were similar, as shown for 4 variants of Der p 2. Conclusion: Two clones for each of the **allergens** were identified as containing sequences that were largely representative of environmental isolates. A small-scale reverse transcriptase PCR used to produce cDNA from individual mites isolated from house **dust** will have wide application for studies on mite genetics and the production of recombinant mite **allergens.** Differences in T-cell responses to peptides representing variant epitopes were found, but responses to variants of whole recombinant **allergens** were similar. The GenBank and Swiss Prot database entries for Der p 1 (U11695) and Der p 2 (P49278) have been updated with

the inclusion of the sequence polymorphisms described in this study.

L32 ANSWER 8 OF 26 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2001:526145 The Genuine Article (R) Number: 445JR. The molecular basis of antigenic cross-reactivity between the group 2 mite **allergens.** Smith A M (Reprint); Benjamin D C; Hozic N; Derewenda U; Smith W A; Thomas W R; Gafvelin G; van Hage-Hamsten M; Chapman M D. Univ Virginia, Hlth Syst, Asthma & Allerg Dis Ctr, POB 801355, Charlottesville, VA 22908 USA (Reprint); Univ Virginia, Hlth Syst, Asthma & Allerg Dis Ctr, Charlottesville, VA 22908 USA; Univ Virginia, Dept Microbiol, Charlottesville, VA 22908 USA; Univ Virginia, Dept Mol Physiol & Biol Phys, Charlottesville, VA 22908 USA; Univ Western Australia, TVW Inst Child Hlth Res, W Perth, WA, Australia; Univ Western Australia, Ctr Child Hlth Res, W Perth, WA, Australia; Karolinska Hosp & Inst, Dept Med, Div Clin Immunol & Allergy, Stockholm, Sweden. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (JUN 2001) Vol. 107, No. 6, pp. 977-984. Publisher: MOSBY, INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318 USA. ISSN: 0091-6749 . Pub. country: USA; Australia; Sweden. Language: English.
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Background: Mite group 2 **allergens** Der p 2, Der f 2, and fur m 2 are 14-kDa proteins of unknown function that share 83% to 85% amino acid sequence identity. Isoforms of the **allergens** within each genus have been identified which differ by 3 or 4 amino acids, but little is known of the influence of group 2 polymorphisms on human IgE antibody binding.

Objective: The purpose of this study was to investigate the importance of interspecies and isoform substitutions on murine mAb and IgE antibody binding and on the molecular structure of the group 2 **allergens.**

Methods: Site-directed mutagenesis was used to incorporate the isoform **amino acid substitutions** onto the Der p 2.0101 sequence. Recombinant **allergens** were expressed and purified from Escherichia coli and used to evaluate antibody binding by enzyme-linked immunosorbent assay (ELISA). Molecular modeling of the tertiary structure was used to analyze structural differences between the various group 2 **allergens.**

Results: The substitution of asparagine for aspartic acid at position 114 restored mAb binding of rDer p 2.0101; the other Der p 2 isoforms and the 3 rDer f 2 isoforms also reacted in the 2-site ELISA. The correlation of IgE binding to the Der p 2 isoforms was excellent and tended to be higher in the isoforms with the asparagine 114 substitution (r(2) = 0.87 vs r(2) = 0.95), rEur m 2.0101 bound to all mAb except 7A1: when compared with rDer p 2 for IgE binding, rEur m 2.0101 gave a correlation coefficient of r(2) = 0.68. Molecular modeling revealed that fur m 2 and the storage mite homologs Lep d 2 and Tyr p 2 retain the tertiary fold of Der p 2. fur m 2 has a conserved surface, whereas Lep d 2 and Tyr p 2 present most of the **amino acid substitutions** on this surface. Lep d 2 and Tyr p 2 did not react with mAb or with sera from patients with IgE to Dermetophagoides species.

Conclusion: The isoform substitutions of rDer p 2 can be distinguished by mAb. The allergenic cross-reactivity between Der p 2, Der f 2, and fur m 2 is a direct result of the conserved antigenic surface, whereas the lack of cross-reactivity with Lep d 2 and Sr p 2 is a result of the multiple substitutions across this surface.

L32 ANSWER 9 OF 26 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2001145726 EMBASE Sequence polymorphisms and antibody binding to the group 2 **dust** mite **allergens.** Smith A.M.; Benjamin D.C.; Derewenda U.; Smith W.A.; Thomas W.R.; Chapman M.D.. Dr. A.M. Smith, University of Virginia Health System, PO Box 801355, Charlottesville, VA 22908-1355, United States. ams3k@virginia.edu. International Archives of Allergy and Immunology 124/1-3 (61-63) 2001.
Refs: 12.
ISSN: 1018-2438. CODEN: IAAIEG. Pub. Country: Switzerland. Language: English. Summary Language: English.

AB Background: The group 2 **allergens** Der p 2, Der f 2 and Eur m 2 are 14-kD proteins with >180% sequence identity. Isoforms within each genus have been identified which differ by 3-4 amino acids. The aim of this study was to investigate the importance of these substitutions to antibody binding. Methods: Recombinant **allergens** were expressed and purified from Escherichia coli. ELISA and skin testing were used to evaluate antibody binding. Molecular modeling of the tertiary structure was preformed to examine the location of substitutions. Results: The three Der f 2 isoforms and two of three of the Der p 2 isoforms reacted with all monoclonal antibodies (mAb). Der p 2.0101, the isoform with aspartate at position 114, bound all mAb except 1D8. Substitution of asparagine for aspartate restored binding of rDer p 2.0101 to mAb 1D8 and increased the correlation coefficient for IgE binding from 0.72 to 0.77. The three Der p 2 isoforms showed comparable skin test reactivity to nDer p 2 and commercial extract. rEur m 2.0101 bound to all mAb except 7A1 and when compared with rDer p 2 for IgE binding, r(2) = of 0.58 (n = 72). Lep d 2 did not react with mAb or with Dermatophagoidesspp. allergic sera. Modeling revealed that Eur m 2, Lep d 2 and Tyr p 2 retain the tertiary fold of Der p 2 and the substitutions are on the surface. Conclusions: mAb could distinguish isoform substitutions. IgE binding showed a good correlation among all isoforms, thus the recombinant **allergens** are useful for diagnosis. Copyright .COPYRGT. 2001 S. Karger AG, Basel.

L32 ANSWER 10 OF 26 MEDLINE on STN

2001061112. PubMed ID: 11054118. Effects of proline mutations in the major house **dust** mite **allergen** Der f 2 on IgE-binding and histamine-releasing activity. Takai T; Ichikawa S; Hatanaka H; Inagaki F; Okumura Y. (Bioscience Research and Development Laboratory, Asahi Breweries, Ltd, Ibaraki, Japan.. toshiro.takai@asahibeer.co.jp) . European journal of biochemistry / FEBS, (2000 Nov) 267 (22) 6650-6. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Der f 2 is the major group 2 **allergen** from house **dust** mite Dermatophagoides farinae and is composed of 129 amino-acid residues. Wild-type and six proline mutants of Der f 2 (P26A, P34A, P66A, P79A, P95A, and P99A) expressed in Escherichia coli were refolded and purified. Formations of intramolecular disulfide bonds in the purified proteins were confirmed correct. The apparent molecular masses analyzed by gel-filtration were 14-15 kDa. The IgE-binding capacity in the sera of seven mite-allergic patients, inhibitory activity for IgE-binding to immobilized wild-type Der f 2, and activity to stimulate peripheral blood basophils to release histamine in two volunteers were analyzed. P95A and P99A, which slightly differed from the wild-type Der f 2 in their CD spectrum, showed reduced IgE-binding, reduced inhibitory activity, and less histamine-releasing activity than the wild-type. P34A also showed reduced allergenicity. Considering that Pro95, Pro99 and Pro34 are closely located in loops at one end of the tertiary structure of Der f 2, we concluded that these loop regions included an IgE-binding site common to all tested patients. P66A showed reduced IgE-binding in two sera out of seven. P26A and P79A showed no reduced allergenicity. However, in immunoblot analysis after SDS/PAGE under reduced conditions, P79A showed no or markedly reduced IgE-binding while the other mutants showed IgE-binding corresponding to that in the assay using correctly refolded proteins. This suggests that Pro79 is involved in refolding of Der f 2. The findings in this study are important for the understanding of the antigenic structure of mite group 2 **allergens** and for manipulation of the **allergens** for specific immunotherapy.

L32 ANSWER 11 OF 26 MEDLINE on STN DUPLICATE 2

2000281698. PubMed ID: 10820288. Threshold signaling of human Th0 cells in activation and anergy: modulation of effector function by altered TCR ligand. Verhoef A; Lamb J R. (Department of Biology, Imperial College of Science, Technology and Medicine, London, United Kingdom. ) Journal of immunology (Baltimore, Md. : 1950), (2000 Jun 1) 164 (11) 6034-40. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States.

Language: English.

AB Molecular interactions between TCR and its natural ligand, in the presence of costimulatory signals, elicit T cell effector functions, whereas subtle changes in the structure of antigenic peptides may induce only selected T cell effector function including anergy. In this study, we have investigated the immunological activity of an altered TCR ligand (p 2, 28-40A34,36) derived from the immunodominant T cell epitope of the group 2 **allergen** of house **dust** mite, in which residues at positions 34 and 36 were substituted by alanine. Elevated IFN-gamma synthesis was induced by equimolar concentrations of the analogue compared with native peptide (p 2, 28-40) and was paralleled by increased down-regulation of cell surface CD3. IL-5 and IL-10 production exhibit the same sensitivity to both peptides, implying that the induction of T cell effector functions are not all proportional to TCR occupancy. Both native peptide and the analogue bound to MHC class II (DRB1*1101) molecules with similar affinities. Furthermore, p 2, 28-40A34,36 induced T cell anergy at lower concentrations than native peptide. During the induction of anergy, TGF-beta production was comparable for both peptides, whereas IL-10 secretion was markedly increased but more so in response to p 2, 28-40A34,36. Membrane expression of costimulatory ligands CD80 and CD86 was similar for native peptide and p 2, 28-40A34,36 and increased in activation, whereas only CD86 was elevated during anergy. The modulation of T cell effector function with altered TCR ligands may have practical applications in reprogramming allergic inflammatory responses through the induction of T cell anergy and/or the promotion of Th1 cytokines.

L32 ANSWER 12 OF 26 MEDLINE on STN

2000437841. PubMed ID: 10946323. C8/119S mutation of major mite **allergen** Derf-2 leads to degenerate secondary structure and molecular polymerization and induces potent and exclusive Th1 cell differentiation. Korematsu S; Tanaka Y; Hosoi S; Koyanagi S; Yokota T; Mikami B; Minato N. (Department of Immunology and Cell Biology, Graduate School of Medicine, and Research Institute for Food Science, Kyoto University, Kyoto, Japan. ) Journal of immunology (Baltimore, Md. : 1950), (2000 Sep 1) 165 (5) 2895-902. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Hyposensitization therapy for atopic diseases has been conducted for decades but suffered from many problems including anaphylactic reactions. We previously developed a mutant protein of the major mite **allergen** Derf-2, C8/119S, which showed reduced binding to IgE. The C8/119S mutant was shown to exhibit more efficient hyposensitizing effect than Derf-2 in the animal model of allergic bronchial asthma. In the present study, we indicate that C8/119S exhibits markedly augmented immunogenicity for the proliferation of Derf-2-specific human T cells and T cell clones irrespective of the epitope specificity as compared with Derf-2. Furthermore, C8/119S has induced potent and almost exclusive differentiation of Th1 cells from the peripheral blood of atopic patients in vitro. Neither Ag dosage effect nor absence of B cell-mediated Ag presentation could fully account for these effects. C8/119S has been indicated to lose the characteristic beta-barrel structure as judged by circular dichroism spectroscopic analysis and to polymerize solubly in physiological condition. Heating of Derf-2 also caused less stable molecular aggregation, but it hardly affected the secondary structure and failed to induce such a polarity toward the Th1 cell differentiation. These results have indicated that the degenerate secondary structure of C8/119S leading to stable molecular polymerization is primarily responsible for the marked increase in T cell-immunogenicity and the induction of exclusive Th1 cell differentiation in atopic patients. It has been suggested strongly that the recombinant C8/119S protein can provide an effective Ag with the least risk of anaphylaxis for **allergen** immunotherapy against house **dust** mite in human.

L32 ANSWER 13 OF 26 EMBASE  on STN

2000405250 EMBASE Immune response of HLA-DQ transgenic mice to house

**dust** mite **allergen** p2: Identification of HLA-DQ restricted minimal epitopes and critical residues. Krco C.J.; Harders J.; Chapoval S.; David C.S.. C.S. David, Department of Immunology, Mayo Clinic, Rochester, MN 55905, United States. david.chella@mayo.edu. Clinical Immunology 97/2 (154-161) 2000.
Refs: 34.
ISSN: 1521-6616. CODEN: CLIIFY. Pub. Country: United States. Language: English. Summary Language: English.

AB HLA-DQ8 (HLA-DQA1*0301; HLA-DQB1*0302) and HLA-DQ6 (HLA-DQA1*0103; HLA-DQB1*0602) genes were introduced into mouse class II (H-2A(β/°)), knockout mice. Transgenic HLA-DQ8 and HLA-DQ6 mice were individually immunized and challenged using synthetic peptides representing HDM (Dermatophagoides pteronyssinus) **allergen** p2. HLA-DQ8 mice responded to p2 peptides 1-20, 41-60, 51-70, 61-80, 91-110, and 101-120. HLA-DQ6 mice responded to peptides 1-20, 11-30, 21-40, 41-60, and 51-70. Using single amino acid truncated 30-mer peptides, residues necessary for HLA-DQ8 recognition were identified spanning regions 3-12, 50-70, and 91-120. A synthetic peptide comprising residues 3-12 was synthesized and a series of single alanine substitutions was introduced into the minimal peptide. Introduction of alanine residues at positions 3, 11, and 12 resulted in a significant loss of immune recognition. It was concluded that residues 4, 5, 7, 11, and 12 are critical for immune recognition by HLA-DQ8 mice. (C) 2000 Academic Press.

L32 ANSWER 14 OF 26 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
1999:157509 The Genuine Article (R) Number: 167JW. T-cell receptor contact and MHC binding residues of a major rye grass pollen **allergen** T-cell epitope. Burton M D; Blaher B; Suphioglu C; OHehir R E; Carbone F R; Rolland J M (Reprint). MONASH UNIV, SCH MED, DEPT PATHOL & IMMUNOL, COMMERCIAL RD, PRAHRAN, VIC 3181, AUSTRALIA (Reprint); MONASH UNIV, SCH MED, DEPT PATHOL & IMMUNOL, PRAHRAN, VIC 3181, AUSTRALIA; ALFRED HOSP, DEPT ALLERGY & CLIN IMMUNOL, PRAHRAN, VIC, AUSTRALIA. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (FEB 1999) Vol. 103, No. 2, Part 1, pp. 255-261. Publisher: MOSBY-YEAR BOOK INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318. ISSN: 0091-6749. Pub. country: AUSTRALIA. Language: English.
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Background: T cells are pivotal in the elicitation of allergic diseases. Analogues of T-cell epitope peptides with a modification at a T-cell receptor (TCR) contact site can alter selected T-cell effector functions. Thus the ability to modulate **allergen**-specific T-cell responses towards T-H1-like by stimulation with peptide analogues may downregulate allergic inflammation.

Objectives: The purpose of this study was to characterize the minimal epitope recognized by cloned T cells of a dominant Lol p 5 epitope, p105-116, and identify the critical residues involved in TCR and MHC contact.

Methods: Using peptides with progressive truncation of N- and C-terminal residues in T-cell proliferation assays, we identified the core epitope recognized by cloned CD4(+) T cells. An additional series of peptides with single **amino acid substitutions** were used in T-cell proliferation and live-cell MHC binding assays. Taken together, these results allowed identification of MHC binding and TCR contact residues of p105-116.

Results: The core epitope of p105-116 was identified as residues 107-114. Within this core epitope, 3 residues were found to be important for MHC binding, positions 107, 110, and 112, whereas those at positions 108, 109, 110, 111, and 113 were putative TCR contact residues.

Conclusions: The identification of the TCR and MHC contact residues of a dominant Lol p 5 T-cell epitope and analogues of this peptide capable of modulating T-cell responses will allow the evaluation of these peptides' potential as immunotherapeutic agents for rye grass pollen allergic disease.

L32 ANSWER 15 OF 26 MEDLINE on STN DUPLICATE 3
1998224476. PubMed ID: 9564806. Antagonistic peptides specifically inhibit

proliferation, cytokine production, CD40L expression, and help for IgE synthesis by Der p 1-specific human T-cell clones. Fasler S; Aversa G; de Vries J E; Yssel H. (Human Immunology Department, DNAX Research Institute for Molecular and Cellular Biology, Palo Alto, Calif, USA. ) Journal of allergy and clinical immunology, (1998 Apr) 101 (4 Pt 1) 521-30. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Allergic disorders are characterized by IgE antibody responses to a multitude of **allergens** as a result of the ability of these antibodies to specifically bind to high-affinity IgE receptors on mast cells and basophils. This interaction results in receptor activation and release of soluble mediators such as histamine and leukotrienes, which cause allergic reactions in various target organs. Because the synthesis of IgE is tightly regulated by cytokines and CD40 ligand (L) interactions, CD4+ helper T cells are obvious targets, with the aim to modulate **allergen**-induced IgE responses. OBJECTIVES: Because of the central role of **allergen**-specific T-helper type 2 (TH2) cells in the pathway leading to IgE synthesis in vitro and in vivo, we have evaluated the possibility of inhibiting **allergen**-induced activation of these cells by using **allergen**-derived peptides that have been modified by single **amino acid substitutions.** METHODS: Three cloned human TH2-like CD4+ T-cell lines, specific for Der p 1, the major **allergen** in house **dust**, were used in this study. Upon activation with Der p 1 or specific Der p 1-derived wild-type peptides, these T-cell clones produce high levels of IL-4 and IL-5 and low levels of interferon-gamma and IL-2, respectively, and furthermore give help to B cells for the production of IgE in vitro. Modified synthetic peptides were generated by the introduction of single **amino acid substitutions** into two different T-cell activation-inducing epitopes on Der p 1. The effects of these modified peptides were studied in Der p 1-induced proliferation, cytokine production, and in vitro IgE production assays. RESULTS: Several substituted Der p 1-derived peptides failed to induce T-cell proliferation, in contrast to the native peptides. In addition, some of these peptides acted as antagonists by strongly inhibiting wild-type peptide-induced proliferation as well as the production of interferon-gamma, IL-2, IL-4, and IL-5, although the production of the latter two cytokines was less affected than that of interferon-gamma, even at a 100-fold molar excess of the antagonistic peptides. In addition, the presence of an excess of each of the antagonistic peptides during the activation of Der p I-specific T-cell clones prevented induction of CD40L expression, resulting in a failure of these cells to give help to B cells for the production of IgE in vitro, even in the presence of exogenous IL-4. CONCLUSIONS: Substitution of certain amino acid residues in immunogenic Der p 1-derived peptides results in the generation of peptides that fail to induce proliferation of Der p 1-specific T-cell clones. In addition, these modified peptides have strong antagonistic activities on Der p 1-induced proliferation, cytokine production, and CD40L expression by **allergen**-specific T-cell clones as well as on T cell-mediated IgE production by B cells. These findings suggest that modified peptides interfere with **allergen**-induced activation of T cells, including the production of cytokines and the expression of surface molecules important for successful T cell-B cell interactions, and may therefore have therapeutic potential by inhibiting the expansion and function of **allergen**-specific TH2 cells.

L32 ANSWER 16 OF 26 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1998:257635 Document No.: PREV199800257635. Antagonistic peptides specifically inhibit proliferation, cytokine production, CD40L expression, and help for IgE synthesis by Der p 1-specific human T-cell clones. Fasler, Stephan; Aversa, Gregoria; De Vries, Jan E.; Yssel, Hans [Reprint author]. INSERM U454, Hopital Arnaud de Villeneuve, 371 Ave. Doyen Gaston Giraud, 34295 Montpellier Cedex, France. Journal of Allergy and Clinical Immunology, (April, 1998) Vol. 10, No. 4 PART 1, pp. 521-530. print.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

AB Background: Allergic disorders are characterized by IgE antibody responses to a multitude of **allergens** as a result of the ability of these antibodies to specifically bind to high-affinity IgE receptors on mast cells and basophils. This interaction results in receptor activation and release of soluble mediators such as histamine and leukotrienes, which cause allergic reactions in various target organs. Because the synthesis of IgE is tightly regulated by cytokines and CD40 ligand (L) interactions, CD4+ helper T cells are obvious targets, with the aim to modulate **allergen**-induced IgE responses. Objectives: Because of the central role of **allergen**-specific T-helper type 2 (TH2) cells in the pathway leading to IgE synthesis in vitro and in vivo, we have evaluated the possibility of inhibiting **allergen**-induced activation of these cells by using **allergen**-derived peptides that have been modified by single **amino acid substitutions.** Methods: Three cloned human TH2-like CD4+ T-cell lines, specific for Der p 1, the major **allergen** in house **dust**, were used in this study. Upon activation with Der p 1 or specific Der p 1-dervived wild-type peptides, these T-cell clones produce high levels of IL-4 and IL-5 and low levels of interferon-gamma and IL-2, respectively, and furthermore give help to B cells for the production of IgE in vitro. Modified synthetic peptides were generated by the introduction of single **amino acid substitutions** into two different T-cell activation-inducing epitopes on Der p 1. The effects of these modified peptides were studied in Der p 1-induced proliferation, cytokine production, and in vitro IgE production assays. Results: Several substituted Der p 1-derived peptides failed to induce T-cell proliferation, in contrast to the native peptides. In addition, some of these peptides acted as antagonists by strongly inhibiting wild-type peptide-induced proliferation as well as the production of interferon-gamma, IL-2, IL-4, and IL-5, although the production of the latter two cytokines was less affected than that of interferon-gamma, even at a 100-fold molar excess of the antagonistic peptides. In addition, the presence of an excess of each of the antagonistic peptides during the activation of Der p I-specific T-cell clones prevented induction of CD40L expression, resulting in a failure of these cells to give help to B cells for the production of IgE in vitro, even in the presence of exogenous IL-4. Conclusions: Substitution of certain amino acid residues in immunogenic Der p 1-derived peptides results in the generation of peptides that fail to induce proliferation of Der p 1-specific T-cell clones. In addition, these modified peptides have strong antagonistic activities on Der p 1-induced proliferation, cytokine production, and CD40L expression by **allergen**-specific T-cell clones as well as on T cell-mediated IgE production by B cells. These findings suggest that modified peptides interfere with **allergen**-induced activation of T cells, including the production of cytokines and the expression of surface molecules important for successful T cell-B cell interactions, and may therefore have therapeutic potential by inhibiting the expansion and function of **allergen**-specific TH2 cells.

L32 ANSWER 17 OF 26 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
1998:722024 The Genuine Article (R) Number: 119VW. Engineering of hypoallergenic mutants of the Brassica pollen **allergen**, Bra r 1, for immunotherapy. Okada T; Swoboda I; Bhalla P L; Toriyama K (Reprint); Singh M B. TOHOKU UNIV, GRAD SCH AGR SCI, LAB PLANT BREEDING & GENET, AOBA KU, 1-1 TSUTSUMIDORI AMAMIYAMACHI, SENDAI, MIYAGI 9818555, JAPAN (Reprint); TOHOKU UNIV, GRAD SCH AGR SCI, LAB PLANT BREEDING & GENET, AOBA KU, SENDAI, MIYAGI 9818555, JAPAN; UNIV MELBOURNE, INST LAND & FOOD RESOURCES, PLANT MOL BIOL & BIOTECHNOL LAB, PARKVILLE, VIC 3052, AUSTRALIA . FEBS LETTERS (4 SEP 1998) Vol. 434, No. 3, pp. 255-260. Publisher: ELSEVIER SCIENCE BV. PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 0014-5793. Pub. country: JAPAN; AUSTRALIA. Language: English.
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB The Brassica pollen **allergen** Bra r 1 belongs to a new family of Ca2+-binding proteins, characterized by the presence of two potential EF-hand calcium-binding domains. Disruption of these EF-hand motifs by

**amino acid substitutions** demonstrated that both domains of Bra r 1 constitute functional Ca2+-binding sites. Calcium-binding deficient mutants displayed significantly reduced IgE-binding activity. Injection of these mutated Bra r 1 variants into a murine model system showed that mouse Ige raised against the mutants recognized native Bra r 1 in Brassica pollen extracts suggesting the potential use of the engineered **allergens** for effective immunotherapy. (C) 1998 Federation of European Biochemical Societies.

L32 ANSWER 18 OF 26 EMBASE 
on STN
1998099883 EMBASE Immune-reactivity of recombinant isoforms of the major house **dust** mite **allergen** Der p 2. Hakkaart G.A.J.; Chapman M.D.; Aalberse R.C.; Van Ree R.. R. Van Ree, Centr. Laboratory of the Netherlands, Red Cross Blood Transfusion Service, Laboratory Exp./Clinic. Immunol., Plesmanlaan 125, 1066 CX Amsterdam, Netherlands. Clinical and Experimental Allergy 28/2 (169-174) 1998.
Refs: 21.
ISSN: 0954-7894. CODEN: CLEAEN. Pub. Country: United Kingdom. Language: English. Summary Language: English.
AB Background: Recombinant Der p 2, expressed in yeast, lacked reactivity with 5 monoclonal antibodies against natural Der p 2. Objective: The aim of this study was to investigate whether the lack of reactivity with recombinant Der p 2 can be explained by the existence of isoforms. Methods: By site- directed mutagenesis three recombinant isoforms of Der p 2 were produced. Reactivity with monoclonal antibodies and human IgE was analysed by means of RAST and RAST-inhibition. Results: All five monoclonals that lacked reactivity with the originally selected isoform, showed reactivity upon replacement of aspartic acid by asparagine at position 114. The other two substitutions (at position 26 and 47) had no effect. Binding of human IgE (n = 10) was not significantly influenced by the isogenetic variation at position 114. Conclusions: Monoclonal antibodies raised against natural Der p 2 can sometimes discriminate between different isoforms, allowing the study of the natural occurrence of isoforms. For application in **allergen**- measurement assays, non-discriminating monoclonal antibodies should be selected.

L32 ANSWER 19 OF 26 MEDLINE on STN DUPLICATE 4
97322946. PubMed ID: 9179436. Localization of antigenic sites on Der p 2 using oligonucleotide-directed mutagenesis targeted to predicted surface residues. Smith A M; Chapman M D. (Department of Medicine, University of Virginia, Charlottesville 22908, USA. ) Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (1997 May) 27 (5) 593-9. Journal code: 8906443. ISSN: 0954-7894. Pub. country: ENGLAND: United Kingdom. Language: English.
AB BACKGROUND: Understanding the molecular nature of **allergen** -antibody interactions is important to understanding the mechanism of conventional immunotherapy as well as to designing alternative immunotherapeutic strategies. Many important **allergens** have been cloned and expressed, making it possible to apply recombinant DNA techniques to dissect antigenic determinants. OBJECTIVE: The aim of this study was to use predictive algorithms and site-directed mutagenesis to investigate monoclonal antibody and IgE antibody epitopes of the major house **dust** mite **allergen** Der p 2. METHODS: Computer algorithms were used to assess the primary amino acid sequence of Der p 2 and to identify regions of hydrophilic and flexible sequence. Subsequently, site-directed mutagenesis was used to generate **amino acid substitutions** at hydrophilic residues at positions 44-46 and at position 100. The variants were tested in a competitive inhibition ELISA with four group 2-specific murine monoclonal antibodies and with human IgE antibody from mite allergic patients. RESULTS: Conservative **amino acid substitutions** at position 44-46 did not distinguish IgE antibody epitopes, but did suggest that these residues are involved in the epitope defined by one monoclonal antibody, 15E11. Non-conservative substitution of proline at this

position reduced binding to all four monoclonal antibodies, as well as IgE antibody, by 50-80%. Point mutants at position 100 mapped the epitopes of two monoclonal antibodies, 7A1 and 13A4, previously shown to bind the same region of Der p 2. In addition, the two variants tested at this position showed distinct inhibition curves with these two monoclonal antibodies indicating differences in fine specificity. CONCLUSIONS: Using predictive algorithms, in the absence of tertiary structural information, we have been able to localize important B cell determinants on Der p 2. The results suggest that it is possible to modulate antibody recognition of **allergens** using site-directed mutagenesis and that this approach may provide a new strategy for **allergen** specific immunotherapy.

L32 ANSWER 20 OF 26 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
96:401493 The Genuine Article (R) Number: UL572. SEQUENCE POLYMORPHISMS OF THE DER-P-3 HOUSE-**DUST** MITE **ALLERGEN.** SMITH W A; THOMAS W R (Reprint). TVW TELETHON INST CHILD HLTH RES, POB 855, PERTH, WA 6872, AUSTRALIA (Reprint); TVW TELETHON INST CHILD HLTH RES, PERTH, WA 6872, AUSTRALIA. CLINICAL AND EXPERIMENTAL ALLERGY (MAY 1996) Vol. 26, No. 5, pp. 571-579. ISSN: 0954-7894. Pub. country: AUSTRALIA. Language: ENGLISH.
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Background The trypsin-like protein Der p 3 is a major **allergen** of Dermatophagoides pteronyssinus. Like other vertebrate and invertebrate trypsin-like molecules, isoelectricfocusing studies with the natural Der p 3 protein have indicated that several isoforms exist.

Objective To determine the extent of the sequence variation of the Der p 3 **allergen** and distinguish at the molecular level, whether the sequence isoforms represent allelic variants or multiple genes of the **allergen.**

Methods Five cDNA clones of Der p 3 have been isolated from a lambda gt10 D. pteronyssinus library, using a radiolabelled polymerase chain reaction (PCR) Der p 3 P3WS1 probe and sequenced. Southern blot and inverse PCR analysis of Eco R1 digested genomic DNA was performed.

Results Southern blot analysis of Eco R1 digested genomic DNA showed that the DNA encoding Der p 3 was located on a single 3.5 kb fragment and inverse polymerase chain reaction analysis (PCR) of this DNA showed that there was only a single Der p 3 gene on this 3.5 kb fragment. The nucleotide sequence of one of the clones was identical to the original Der p 3 P3WS1 clone and two clones differed only in their 3' untranslated sequences. The other two contained nucleotide changes which lead to several substitutions at the amino acid level, both conservative and non conservative. Clone 3 had 98.7% identity with Der p 3 P3WS1. One clone for which the full sequence was not available (clone 4) had only 84.4% identity with the original clone and is therefore consistent with an isoallergen.

Conclusions These data along with our previous genomic sequence shows that for the most part, the Der p 3 **allergen** has only minor sequence variations (variants) although the isoallergen indicated by clone 4 needs further investigation. It is now evident that Der p 3 is encoded by a single gene and that most cDNA clones constructed from commercial mites show only minor sequence variation similar to that observed for the group 1 and group 2 house **dust** mite **allergens.**

L32 ANSWER 21 OF 26 MEDLINE on STN
96435212. PubMed ID: 8838098. Molecular biological analysis of house **dust** mite **allergens.** Okudaira H; Okumura Y; Sato G. (Department of Medicine and Physical Therapy, University of Tokyo. ) Nippon rinsho. Japanese journal of clinical medicine, (1996 Feb) 54 (2) 466-71. Ref: 10. Journal code: 0420546. ISSN: 0047-1852. Pub. country: Japan. Language: Japanese.

AB Using a host-vector system of Escherichia coli, we could produce one of major house **dust** mite **allergens**, Der f II in large quantity for therapeutic and diagnostic purposes. About 5 mg of purified and biologically active rDer f II was obtained from one liter culture, which was corresponding to the amount in about 30 g of live mite. The

rDer f II was almost identical with native Der f II with respects to biological and physicochemical view points. Native mite Der f II is a mixture of several kinds of Der f II molecule with a few **amino acid substitutions**, which were due to polymorphisms among individual mite gene sequence. We had cloned three kinds of Der f II cDNAs from mite culture and expressed in E. coli and prepared three kinds of rDer f II in this system. As a result of comparison of IgE binding activity among three rDer f IIs and nDer f II, there was no significant difference observed.

L32 ANSWER 22 OF 26 MEDLINE on STN DUPLICATE 5

96319509. PubMed ID: 8768806. IgE responsiveness to Dermatophagoides farinae in young asthmatic children: IgE binding study using recombinant **allergens** of Der f1, Der f2 and mutant proteins of Der f2. Noguchi E; Shibasaki M; Nishiyama C; Okumura Y; Takita H. (Department of Pediatrics, Institute of Clinical Medicine, University of Tsukuba, Japan. ) International archives of allergy and immunology, (1996 Aug) 110 (4) 380-7. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB IgE reactivities to two recombinant (r) **allergens**, rDer f1 and rDer f2, synthetized by cDNA clones from Dermatophagoides farinae (Df) were analyzed using sera of Df-sensitive asthmatic patients of varying ages. Positive RAST responses to rDer f1 and rDer f2 were found in 88 and 80%, respectively, with sera from young asthmatic children aged 0-1, positive RAST rates to these antigens increased to 100% up to 5 years of age. There was a significant correlation between RAST levels of rDer f1 and rDer f2 in these young children aged 0-1. IgE reactivities to four mutant proteins of rDer f2, which have at least one **amino acid substitution**, were similarly examined in the asthmatic sera. IgE reactivity to D7A, in which Asp7 was replaced by Ala, was reduced to 30-60% compared to the rDer f2. IgE binding to C8/119S, in which both Cys8 and Cys119 were replaced by Ser, lacking a disulfide bond between Cys8 and Cys119, was reduced almost to a background level at all ages. In contrast, A72L and A120L, in which Ala72 and Ala120 were substituted for Leu, respectively, almost retained the same IgE binding activity as the rDer f2 at all ages. These results suggest that Der f1 and Der f2 are important antigens associated with early sensitization to house **dust** mite in young asthmatic children. In addition, the disulfide bond between Cys8 and Cys119 and the N-terminal region including the 7th amino acid residue are considered to maintain IgE epitopes of the Der f2 **allergen.**

L32 ANSWER 23 OF 26 MEDLINE on STN DUPLICATE 6

96162074. PubMed ID: 8563488. Comparative analysis of the genes encoding group 3 **allergens** from Dermatophagoides pteronyssinus and Dermatophagoides farinae. Smith W A; Thomas W R. (TVW Telethon Research Institute for Child Health, Perth, Australia. ) International archives of allergy and immunology, (1996 Feb) 109 (2) 133-40. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB Group 3 **allergens** of the genus Dermatophagoides represent one of the major groups of house **dust** mite **allergens.** The cDNA sequence data for Der p 3, in combination with both N-terminal amino acid sequences and substrate affinity data, have confirmed that the group 3 **allergens** are trypsin-like proteases. Using the information from the Der p 3 P3WS1 cDNA clone, genes encoding both Der f3 and Der p 3 have now been amplified by the polymerase chain reaction and analysed. Two Der f3 clones and three Der p 3 genomic clones were sequenced. Each of the clones contained a single small intron and encoded a mature protein of 233 amino acids. The nucleotide sequence was identical for both Der f3 clones. There was 81% identity between the Der f3 sequence and the original Der p 3 P3WS1 clone. The calculated molecular weight of Der f3 was 25.27 kDa compared to 24.98 kDa for Der p 3. All the amino acid residues required for the catalytic activity and the substrate specificity were conserved between the two homologues. The coding sequences of two of the three Der p 3 genomic clones were identical to the original Der p 3

P3WS1 clone with the third having nucleotide changes resulting in four non-conservative **amino acid substitutions** in the mature protein. These substitutions resulted in a molecule with a slightly larger molecular weight and a more acidic pI value than the original Der p 3 clone. This third Der p 3 genomic clone is, therefore, an isoform of the Der p 3 P3WS1 clone and is classified as an isovariant of the **allergen.** The nucleotide sequence data presented are the first reported for Der f 3. The Der f 3 gene, like the Der p 3 gene, encoded a trypsin-like protease, but with a slightly larger molecular weight.

L32 ANSWER 24 OF 26 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
96:110697 The Genuine Article (R) Number: TR947. SINGLE **AMINO-ACID SUBSTITUTIONS** ON A JAPANESE CEDAR POLLEN **ALLERGEN** (CRY-J-1)-DERIVED PEPTIDE-INDUCED ALTERATIONS IN HUMAN T-CELL RESPONSES AND T-CELL RECEPTOR ANTAGONISM. IKAGAWA S; MATSUSHITA S; CHEN Y Z; ISHIKAWA T; NISHIMURA Y (Reprint). KUMAMOTO UNIV, GRAD SCH MED SCI, DEPT NEUROSCI & IMMUNOL, DIV IMMUNOGENET, HONJO 2-2-1, KUMAMOTO 860, JAPAN (Reprint); KUMAMOTO UNIV, GRAD SCH MED SCI, DEPT NEUROSCI & IMMUNOL, DIV IMMUNOGENET, / KUMAMOTO 860, JAPAN; KUMAMOTO UNIV, SCH MED, DEPT OTORHINOLARYNGOL, KUMAMOTO 860, JAPAN. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (JAN 1996) Vol. 97, No. 1, Part 1, pp. 53-64. ISSN: 0091-6749. Pub. country: JAPAN. Language: ENGLISH.
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB We generated T cell clones specific to a Japanese cedar pollen **allergen** (Cry j 1) and investigated effects of altered T cell receptor (TCR) ligand on changes of T cell responses. One of these Cry j 1-specific T cell clones established from patients with Japanese cedar pollinosis, ST1.9, recognized an antigenic peptide Cry j 1 p335-346 in the contest of HLA-DRA +DRB3*0301 molecules and secreted interleukin-4 dominantly, with a smaller amount of interferon-gamma. ST1.9 represented one of the major T cell clones specific to Cry j 1 in the donor, because a short-term cultured polyclonal T cell line specific to Cry j 1 cuhibited the same character as the ST1.9. We synthesized various analog peptides derived from Cry j 1 p335-346 with single **amino acid substitutions** and determined key residues for interactions between TCR of ST1.9 and HLA-DR molecules. We also analyzed changes in the responses of ST1.9 to Cry j 1 p335-346-derived analog peptides. Of interest was that the substitution of (339)threonine to valine resulted in a significant increase in interferon-gamma production, with no remarkable changes either ill proliferative response or interleukin-4 production. Analog peptides carrying the substitutions of (339)threonine to glycine ol glutamine revealed TCR antagonism, without changes in their binding affinities to the DR molecule. Therefore single **amino acid substitutions** on an **allergen** peptide carrying the T cell epitope may suppress helper-T-dependent class switch pressure to IgE in B cells either by inducing increased interferon-gamma production or by inhibiting proliferative responses in helper-T cells.

L32 ANSWER 25 OF 26 CAPLUS COPYRIGHT 2004 ACS on STN
1993:579195 Document No. 119:179195 T cell epitopes of the major **allergens** from Dermatophagoides (house **dust** mite).
Garman, Richard D.; Greenstein, Julia L.; Kuo, Mei Chang; Rogers, Bruce L. (Immulogic Pharmaceutical Corp., USA). PCT Int. Appl. WO 9308279 A1 19930429, 176 pp. DESIGNATED STATES: W: AU, CA, FI, HU, JP, KR, NO; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1992-US8637 19921015. PRIORITY: US 1991-777859 19911016; US 1992-881396 19920508.

AB The 4 **allergens** were immunoaffinity-purified from spent mite culture media. Recombinant **allergens** were also prepared by cloning and expressing the cDNA in BL21 cells; amino acid sequence polymorphisms were discovered. T cell epitopic studies and cross reactivity studies are shown. There was no detectable IgE reactivity to any of 56 T cell epitopic peptides screened.

L32 ANSWER 26 OF 26 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
93250515 EMBASE Document No.: 1993250515. Heterogeneity in the IgE binding to a peptide derived from the house **dust** mite **allergen**
Der p II indicates that the IgE response is highly polyclonal. Van't Hof W.; Van den Berg M.; Driedijk P.C.; Aalberse R.C.. Publication Secretariate, Central Laboratory, Netherlands Red Cross Blood Trans Se, P.O. Box 9406,NL-1006 AK Amsterdam, Netherlands. International Archives of Allergy and Immunology 101/4 (437-441) 1993.
ISSN: 1018-2438. CODEN: IAAIEG. Pub. Country: Switzerland. Language: English. Summary Language: English.
AB The fine specificity of IgE antibody binding to peptide 65-78 of the house **dust** mite major **allergen** Der p II was examined by comparison with binding to two peptides in which the cysteines corresponding to cys73 and cys78 in Der p II were substituted by serines and methionines. Differences in binding behavior indicated that at least three different subpopulations of IgE antibodies bound to peptide 65-78. Even at the level of such a small fragment the IgE response in individual donors proved to be polyclonal.

=> s l12 and grass
L33 25 L12 AND GRASS

=> dup remove l33
PROCESSING COMPLETED FOR L33
L34 9 DUP REMOVE L33 (16 DUPLICATES REMOVED)

=> d l34 1-9 cbib abs

L34 ANSWER 1 OF 9 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2003518045 EMBASE Effect of cysteine mutagenesis on human IgE reactivity of recombinant forms of the major rye **grass** pollen **allergen**
Lol p 1. De Weerd N.; Bhalla P.L.; Singh M.B.. Prof. M.B. Singh, Plant Molec. Biol. and Biotech. Lab., Institute of Land and Food Resources, University of Melbourne, Parkville, Vic. 3010, Australia. m.singh@landfood.unimelb.edu.au. Allergology International 52/4 (183-190) 2003.
Refs: 36.
ISSN: 1323-8930. CODEN: ALINFR. Pub. Country: Australia. Language: English. Summary Language: English.
AB Background: Hypersensitivity to the group 1 **grass** pollen **allergens** is a significant causative factor in the onset of symptoms for hay fever sufferers. To better understand the IgE reactivity of the group 1 **allergen** from rye **grass** pollen, we sought to disrupt potential conformational IgE epitopes on recombinant (r) Lol p 1 by the specific replacement of the seven cysteine residues in the protein sequence. Methods: Site-directed mutagenesis on the Lol p 1 coding sequence was used to replace all seven cysteine residues with serine residues, rLol p 1 and the seven cysteine variants generated by this method were tested for comparative human IgE reactivity via western blot immunoscreening and densitometry. Results: Alteration of the cysteine residues at amino acid positions 72, 77, 83 and 139 of rLol p 1 was found to reduce the human IgE binding potential of the molecule. However, the most consistent reduction in human IgE reactivity was demonstrated by replacement of C (77); human IgE antibodies showed an average 62.7% reduction in reactivity to this molecule. Conclusions: The present investigation has shown that at least one of the cysteine residues within the Lol p 1 protein contributes to the IgE binding properties of this **allergen.**

L34 ANSWER 2 OF 9 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2000:758 The Genuine Article (R) Number: 266FD. Molecular and immunologic characterization of new isoforms of the Hevea brasiliensis latex

**allergen** Hev b 7: Evidence of no cross-reactivity between Hev b 7 isoforms and potato patatin and proteins from avocado and banana. Sowka S; Hafner C; Radauer C; Focke M; Brehler R; Astwood J D; Arif S A M; Kanani A; Sussman G L; Scheiner O; Beezhold D H; Breiteneder H (Reprint). UNIV HOSP VIENNA, DEPT GEN & EXPT PATHOL, AKH EBO 3Q, WAEHRINGER GUERTEL 18-20, A-1090 VIENNA, AUSTRIA (Reprint); UNIV VIENNA, DEPT GEN & EXPT PATHOL, VIENNA, AUSTRIA; UNIV MUNSTER, DEPT DERMATOL & VENEROL, D-4400 MUNSTER, GERMANY; MONSANTO CO, ST LOUIS, MO; RUBBER RES INST MALAYSIA, KUALA LUMPUR, MALAYSIA; UNIV TORONTO, DEPT ALLERGY & CLIN IMMUNOL, TORONTO, ON, CANADA; GUTHRIE RES INST, SAYRE, PA. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (DEC 1999) Vol. 104, No. 6, pp. 1302-1310. Publisher: MOSBY-YEAR BOOK INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318 . ISSN: 0091-6749. Pub. country: AUSTRIA; GERMANY; USA; MALAYSIA; CANADA. Language: English.

*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Background: Hev b 7 is a Hevea brasiliensis latex **allergen** with sequence identities of 39% to 42% to patatins recently identified as potato **allergens.** The complementary DNAs encoding 2 different Hev b 7 isoforms were previously reported.

Objective: The aim of this study was to determine the sequence variation of Hev b 7 and to compare the IgE reactivity of individual isoforms in vitro and in vivo. A further objective was to evaluate possible cross-reactivities between Hev b 7 and patatins and proteins from banana and avocado.

Methods: An H brasiliensis lambda ZAP complementary DNA (cDNA) Library was screened with use of a Hev b 7 cDNA probe. Four Hev b 7 isoforms Here produced in recombinant form and their IgE-binding capacities a ere compared. IgE immunoblot inhibitions and ELISA inhibition assays were used to investigate the possible cross-reactivity between Hev b 7 and recombinant potato patatin and proteins from avocado and banana.

Results: Two new isoforms, S2 and D-2, were identified by sequencing 32 cDNA clones with full-length coding regions. All 4 recombinant isoforms displayed esterase activity and identical IgE-binding capacities. The new isoforms S2 and D-2 were evaluated in skin prick tests and provoked responses equivalent to natural Hev b 7, No cross-reactivity was observed between Hev b 7 isoforms and potato patatin and proteins from avocado and banana.

Conclusions: All 4 recombinant Hev b 7 Isoforms have equivalent IgE-binding capacity and therefore represent suitable reagents for the development of in vitro and in vivo diagnostic tests. Hev b 7, patatins, and their homologs appear not to contribute to cross-reactivity in the Inter-fruit syndrome.

L34 ANSWER 3 OF 9 MEDLINE on STN DUPLICATE 1

1999220064. PubMed ID: 10202363. Sequence polymorphism of the group 1 **allergen** of Bermuda **grass** pollen. Chang Z N; Peng H J; Lee W C; Chen T S; Chua K Y; Tsai L C; Chi C W; Han S H. (Institute of Biotechnology in Medicine, National Yang-Ming University, Taipei, Taiwan, Republic of China. ) Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (1999 Apr) 29 (4) 488-96. Journal code: 8906443. ISSN: 0954-7894. Pub. country: ENGLAND: United Kingdom. Language: English.

AB BACKGROUND: Cyn d 1, the major **allergen** of Bermuda **grass** pollen, consists of a number of isoforms. OBJECTIVE: To examine the extent of sequence variation of Cyn d 1 isoforms at the molecular level. METHODS: A Bermuda **grass** pollen lambdaZAP II cDNA expression library was immunoscreened with anti-Cyn d 1 monoclonal antibodies. The reactive clones were isolated, subcloned into Escherichia coli, and sequenced. Some of them were expressed in the yeast Pichia pastoris to obtain recombinant Cyn d 1 proteins. RESULTS: Ten cDNA clones were obtained, all these clones encode the full length of Cyn d 1 protein. Their deduced mature proteins can be grouped into: the long ones with 246 amino acids, and the short ones with 244 amino acids. The last two amino acids (AG) of the long Cyn d 1 are deleted in the short Cyn d 1. The remaining amino acid sequences share more than 98% identity; a total of

nine amino acid variations were observed. Two recombinant Cyn d 1 proteins (rCyn d 3-2 and rCyn d 5-4) with three **amino acid substitutions** showed differential IgE-binding profiles. CONCLUSION: The present study extended our understanding of the primary structure of isoforms of Cyn d 1.

L34 ANSWER 4 OF 9 MEDLINE on STN DUPLICATE 2
1999135992. PubMed ID: 9949316. T-cell receptor contact and MHC binding residues of a major rye **grass** pollen **allergen** T-cell epitope. Burton M D; Blaher B; Suphioglu C; O'Hehir R E; Carbone F R; Rolland J M. (Department of Pathology & Immunology, Monash University Medical School, Victoria, Australia. ) Journal of allergy and clinical immunology, (1999 Feb) 103 (2 Pt 1) 255-61. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: T cells are pivotal in the elicitation of allergic diseases. Analogues of T-cell epitope peptides with a modification at a T-cell receptor (TCR) contact site can alter selected T-cell effector functions. Thus the ability to modulate **allergen**-specific T-cell responses towards TH1 -like by stimulation with peptide analogues may downregulate allergic inflammation. OBJECTIVES: The purpose of this study was to characterize the minimal epitope recognized by cloned T cells of a dominant Lol p 5 epitope, p105-116, and identify the critical residues involved in TCR and MHC contact. METHODS: Using peptides with progressive truncation of N- and C-terminal residues in T-cell proliferation assays, we identified the core epitope recognized by cloned CD4(+) T cells. An additional series of peptides with single **amino acid substitutions** were used in T-cell proliferation and live-cell MHC binding assays. Taken together, these results allowed identification of MHC binding and TCR contact residues of p105-116. RESULTS: The core epitope of p105-116 was identified as residues 107-114. Within this core epitope, 3 residues were found to be important for MHC binding, positions 107, 110, and 112, whereas those at positions 108, 109, 110, 111, and 113 were putative TCR contact residues. CONCLUSIONS: The identification of the TCR and MHC contact residues of a dominant Lol p 5 T-cell epitope and analogues of this peptide capable of modulating T-cell responses will allow the evaluation of these peptides' potential as immunotherapeutic agents for rye **grass** pollen allergic disease.

L34 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
1998:682138 Document No. 129:301697 Mutants of **grass allergens** not recognized by IgE of allergic patients and their use in specific immunotherapy. Kahlert, Helga; Stuwe, Hans-Thomas; Fiebig, Helmut; Cromwell, Oliver; Becker, Wolf-Meinhard; Bufe, Albrecht; Schramm, Gabriele; Jager, Lothar; Muller, Wolf-Dieter (Merck Patent G.m.b.H., Germany). PCT Int. Appl. WO 9843657 A2 19981008, 58 pp. DESIGNATED STATES: W: HU, JP, PL, US; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (German). CODEN: PIXXD2. APPLICATION: WO 1998-EP1507 19980316. PRIORITY: DE 1997-19713001 19970327.

AB Mutants of **allergens** of a **grass** (Phleum pratense) that stimulate the lymphocyte proliferation and cytokine synthesis in sufferers of pollen allergies, but have significantly lower binding to serum IgE antibodies of patients are described. The **allergens** can be manufactured by expression of the cloned gene for use in immunotherapy of **grass** allergies. Specifically, the T-cell epitopes of the **allergens** are modified and the modification may arise from a spontaneous mutation or by site-specific mutagenesis. T cell epitopes of the Phl p 5 **allergen** were identified and **allergen** derivs. lacking the most significant ones were prepared by site-directed mutagenesis involving **amino acid substitutions** and deletions. The derivs. showed very little **allergen** activity as judged by their inability to inhibit IgE binding to wild-type **allergen.**

L34 ANSWER 6 OF 9 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
1998:722024 The Genuine Article (R) Number: 119VW. Engineering of

hypoallergenic mutants of the Brassica pollen **allergen**, Bra r 1, for immunotherapy. Okada T; Swoboda I; Bhalla P L; Toriyama K (Reprint); Singh M B. TOHOKU UNIV, GRAD SCH AGR SCI, LAB PLANT BREEDING & GENET, AOBA KU, 1-1 TSUTSUMIDORI AMAMIYAMACHI, SENDAI, MIYAGI 9818555, JAPAN (Reprint); TOHOKU UNIV, GRAD SCH AGR SCI, LAB PLANT BREEDING & GENET, AOBA KU, SENDAI, MIYAGI 9818555, JAPAN; UNIV MELBOURNE, INST LAND & FOOD RESOURCES, PLANT MOL BIOL & BIOTECHNOL LAB, PARKVILLE, VIC 3052, AUSTRALIA . FEBS LETTERS (4 SEP 1998) Vol. 434, No. 3, pp. 255-260. Publisher: ELSEVIER SCIENCE BV. PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 0014-5793. Pub. country: JAPAN; AUSTRALIA. Language: English.
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB The Brassica pollen **allergen** Bra r 1 belongs to a new family of Ca2+-binding proteins, characterized by the presence of two potential EF-hand calcium-binding domains. Disruption of these EF-hand motifs by **amino acid substitutions** demonstrated that both domains of Bra r 1 constitute functional Ca2+-binding sites. Calcium-binding deficient mutants displayed significantly reduced IgE-binding activity. Injection of these mutated Bra r 1 variants into a murine model system showed that mouse Ige raised against the mutants recognized native Bra r 1 in Brassica pollen extracts suggesting the potential use of the engineered **allergens** for effective immunotherapy. (C) 1998 Federation of European Biochemical Societies.

L34 ANSWER 7 OF 9 MEDLINE on STN DUPLICATE 3

97337943. PubMed ID: 9194614. Comparison of natural and recombinant isoforms of **grass** pollen **allergens.** Petersen A; Grobe K; Lindner B; Schlaak M; Becker W M. (Forschungszentrum Borstel, Germany. ) Electrophoresis, (1997 May) 18 (5) 819-25. Journal code: 8204476. ISSN: 0173-0835. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB More than 95% of **grass** pollen allergic patients possess IgE antibodies against **grass** group I, a heterogeneous group of glycoproteins found in all temperate **grasses.** We studied the structural variability of the group I **allergens** in single species and among different **grasses.** By 2-DE blotting using patients' IgE and monoclonal antibodies, we detected IgE-reactive isoforms with molecular masses between 32 and 37 kDa and focusing in a wide pI ranging from 4.7 to 7.6. While the group I **allergens** of timothy **grass** (Phl p 1) were composed of 37 and 35 kDa components, only single isoforms were found for ryegrass (Lol p 1) and velvet **grass** (Hol l 1): 32 and 34 kDa, respectively. By N-terminal microsequencing we determined single **amino acid substitutions** in different-sized group I **allergens.** The post-translational modifications (one N-glycosylation site, two hydroxylated proline residues and seven cysteine residues for potential disulfide formations), which contribute to IgE reactivity, were identical in all. From the cDNA sequences we deduced protein sequence homologies > 90%, a result which might explain the high IgE cross-reactivity among the **grasses.** In order to test whether recombinant group I **grass allergens** can act as substitutes for the natural forms, we expressed rPhl p 1 in E. coli and in P. pasteuris. 2-DE immunoblotting again demonstrated a microheterogeneity in molecular mass and pI. While the E. coli products were free from post-translational modifications, rPhl p 1 from Pichia is a heterogeneous glycoprotein fraction with a carbohydrate content of about 15%. This rPhl p 1 is hyperglycosylated compared to the nPhl p 1, which only has a 5% carbohydrate content.

L34 ANSWER 8 OF 9 MEDLINE on STN DUPLICATE 4

97163424. PubMed ID: 9010244. Human recombinant antibody fragments specific for a rye-**grass** pollen **allergen**: characterization and potential applications. de Lalla C; Tamborini E; Longhi R; Tresoldi E; Manoni M; Siccardi A G; Arosio P; Sidoli A. (Department of Biological and Technological Research, San Raffaele Scientific Institute, Milano, Italy. ) Molecular immunology, (1996 Sep) 33 (13) 1049-58. Journal code: 7905289. ISSN: 0161-5890. Pub. country:

ENGLAND: United Kingdom. Language: English.

AB One of the major **allergens** from the pollen of perennial rye **grass** (Lolium perenne), Lol pII, was used to isolate specific antibody fragments from a random combinatorial library displaying a large repertoire of human Fab on filamentous phages. After five panning cycles on recombinant Lol pII immunotubes, phage binders were isolated and the antibody fragments expressed as soluble Fab molecules in the Escherichia coli periplasm. The DNA sequencing of the clones producing antibodies with the highest binding activity showed three of them to be identical, while one differed by two **amino acid substitutions** in the heavy chain. The antibody fragments were produced in milligram amounts, affinity-purified and further characterized. They bound the natural **allergen** as well as the recombinant one, with no cross-reactivity with other **allergens** contained in the pollen extract of L. perenne. One antibody bound the **allergen** with Kd = 2.63 x 10(-9) M, as demonstrated by the surface plasmon resonance technique, and was able to compete with a fraction of serum IgE. Epitope mapping using synthetic peptides revealed that antigenic domains, located between amino acids 39 and 51 of Lol pII, are recognized by Fab and polyclonal IgE from sera of allergic donors. The Fab fragments inhibited the binding of serum IgE to the **allergen**. In vitro experiments on whole blood from allergic subjects showed that recombinant Fab fragments had a blocking activity on histamine release from cells challenged with recombinant Lol pII **allergen.** Thus, serum IgE and recombinant Fab fragments recognize common epitopes, although they represent the outcome of different maturation and/or selection processes. Our molecular and functional findings altogether indicate that **allergen**-specific human antibodies may be useful for the characterization of the antigenic structure of **allergens**. We conclude that a phage library is a powerful source of anti-**allergen** human antibodies with high affinity and high specificity. Moreover, these molecules may be potentially innovative reagents for the treatment of atopic allergy.

L34 ANSWER 9 OF 9 EMBASE 
on STN

97099625 EMBASE Document No.: 1997099625. Characterization of group 1 **allergens** from eleven **grass** species. Aasmul-Olsen S.; Wurtzen P.A.; Lombardero M.; Lowenstein H.; Ipsen H.. S. Aasmul-Olsen, ALK-ABELLO Group, Boge Alle 10-12, 2970 Horsholm, Denmark. Advances in Experimental Medicine and Biology 409/- (261-265) 1996.
Refs: 27.
ISSN: 0065-2598. CODEN: AEMBAP. Pub. Country: United States. Language: English.

```
=> s l12 and tree
L35         13 L12 AND TREE

=> dup remove l35
PROCESSING COMPLETED FOR L35
L36          6 DUP REMOVE L35 (7 DUPLICATES REMOVED)

=> d l36 1-6 cbib abs
```

L36 ANSWER 1 OF 6 MEDLINE on STN DUPLICATE 1

2002095800. PubMed ID: 11799381. Linear IgE epitope mapping of the English walnut (Juglans regia) major food **allergen**, Jug r 1. Robotham Jason M; Teuber Suzanne S; Sathe Shridhar K; Roux Kenneth H. (Department of Biological Science and Structural Biology Program, Florida State University, Tallahassee 32306-4370, USA. ) Journal of allergy and clinical immunology, (2002 Jan) 109 (1) 143-9. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Peanut and **tree** nut allergies can be life-threatening, and they appear to be growing in prevalence. Jug r 1, a

2S albumin seed storage protein, was previously characterized as a major English walnut food **allergen.** OBJECTIVE: We sought to identify the linear IgE-binding epitopes of Jug r 1 and to determine which, if any, amino acids are necessary for this binding to occur. METHODS: Pools of sera from walnut-allergic patients and overlapping peptides synthesized on an activated cellulose membrane were used to screen for IgE-binding epitopes. Mutational analysis of the immunodominant epitope was carried out through single and multisite **amino acid substitutions.** Inhibition assays were performed through use of affinity-purified IgE, soluble forms of the epitope peptide, and the recombinant 2S albumin, rJug r 1. RESULTS: One immunodominant linear epitope was identified. Amino acid mutations to the epitope demonstrated that the residues RGEE, at positions 36 through 39, were minimally required for IgE binding. Probing of this epitope with sera from each of 20 patients revealed 15 of the sera to be positive. Binding of patients' IgE to the epitope was inhibited with a soluble form of the peptide; however, soluble peptide did not completely inhibit the binding of IgE to the intact rJug r 1. CONCLUSION: One major linear IgE-reactive epitope and its critical core amino acid residues have been identified. Mutation of any of these core amino acids resulted in loss of IgE binding to the epitope, and this points toward the feasibility of reducing allergenicity in genetically modified walnuts. However, strong evidence for the existence of conformational epitopes was also obtained.

L36 ANSWER 2 OF 6 MEDLINE on STN

2002014527. PubMed ID: 11419722. Molecular basis of allergic cross-reactivity between group 1 major **allergens** from birch and apple. Holm J; Baerentzen G; Gajhede M; Ipsen H; Larsen J N; Lowenstein H; Wissenbach M; Spangfort M D. (Biochemical Allergy Research, ALK-Abello A/S, Horsholm, Denmark. ) Journal of chromatography. B, Biomedical sciences and applications, (2001 May 25) 756 (1-2) 307-13. Journal code: 9714109. ISSN: 1387-2273. Pub. country: Netherlands. Language: English.

AB Patients allergic to birch pollen often also react with fruits and vegetables, such as apple. The major cause of cross-reactivity between birch and apple is biochemical and immunological similarity between the major **allergens**, Bet v 1 and Mal d 1, as demonstrated by serological and cellular immunoassays. In addition, birch pollen-specific therapeutic allergy vaccination has been shown to improve allergic symptoms caused by oral ingestion of apple. Detailed analysis of molecular surface areas based on the crystal structure of Bet v 1, and primary sequence alignment, identify potential epitopes for cross-reactive antibodies. Two or more conserved patches are identified when comparing Bet v 1 and Mal d 1, thus providing a molecular model for serological cross-reactivity involving more than one IgE-binding epitope. A minimum of two epitopes would be necessary for cross-linking of receptor bound IgE in functional histamine release assays and skin test. Individual **amino acid substitutions**, as occurring in isoallergenic variation, may, however, have a dramatic effect on epitope integrity if critical residues are affected. Thus, one area large enough to accommodate antibody-binding epitopes shared by all known Mal d 1 isoallergens and variants is identified, as well as areas shared by Bet v 1 and individual Mal d 1 isoallergens or variants. The occurrence of limited epitope coincidence between Bet v 1 and Mal d 1 is in agreement with the observation that some, but not all, birch pollen allergic patients react with apple, and that the epitope repertoire recognised by the IgE of the individual patients determines the degree of cross-reactivity.

L36 ANSWER 3 OF 6 EMBASE  on STN

1999229979 EMBASE The structure of major birch pollen **allergens** - Epitopes, reactivity and cross-reactivity. Spangfort M.D.; Mirza O.; Holm J.; Larsen J.N.; Ipsen H.; Lowenstein H.. M.D. Spangfort, Biochemical Allergy Research, ALK-Abello, Boge Alle 6-8, DK-2970 Horsholm, Denmark. Allergy: European Journal of Allergy and Clinical Immunology, Supplement

54/50 (23-26) 1999.
Refs: 5.
ISSN: 0108-1675. CODEN: ALSUET. Pub. Country: Denmark. Language: English.

L36 ANSWER 4 OF 6 MEDLINE on STN
97249364. PubMed ID: 9095250. Characterisation of recombinant isoforms of birch pollen **allergen** Bet v 1. Spangfort M D; Ipsen H; Sparholt S H; Aasmul-Olsen S; Osmark P; Poulsen F M; Larsen M; Mortz E; Roepstorff P; Larsen J N. (ALK Laboratories, Horsholm, Denmark. ) Advances in experimental medicine and biology, (1996) 409 251-4. Journal code: 0121103. ISSN: 0065-2598. Pub. country: United States. Language: English.
AB Three isoforms of the major birch pollen **allergen**, Bet v, 1 from Betula verrucosa have been expressed as recombinant proteins in E. coli and purified. The immunochemical properties of recombinant isoforms (rBet v 1) differed on immunoblots when compared using Mabs and birch pollen allergic patients serum IgE. 2-D gel analysis showed that recombinant isoforms with different epitope structure can focus under the same protein spot after electrophoresis. The structure of conformational epitopes can be distorted by **amino acid substitutions** even when T-cell epitopes are not affected as judged by T-cell proliferation studies.

L36 ANSWER 5 OF 6 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 2
97099605 EMBASE Document No.: 1997099605. Modulation of IgE-binding properties of **tree** pollen **allergens** by site- directed mutagenesis. Ferreira F.; Rohlfs A.; Hoffmann-Sommergruber K.; Schenk S.; Ebner C.; Briza P.; Jilek A.; Kraft D.; Breitenbach M.; Scheiner O.. F. Ferreira, Inst. f. Genetik u. Allg. Biologie, Universitat Salzburg, A-5020 Salzburg, Austria. Advances in Experimental Medicine and Biology 409/- (127-135) 1996.
Refs: 13.
ISSN: 0065-2598. CODEN: AEMBAP. Pub. Country: United States. Language: English.

L36 ANSWER 6 OF 6 MEDLINE on STN DUPLICATE 3
95337757. PubMed ID: 7542079. The significance of isoallergenic variations in present and future specific immunotherapy. Lowenstein H; Sparholt S H; Klysner S S; Ipsen H; Larsen J N. (ALK Laboratories, Horsholm, Denmark. ) International archives of allergy and immunology, (1995 May-Jun) 107 (1-3) 285-9. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.
AB The isoallergenic variation of the **tree** pollen major **allergens** has been studied by 2D gel electrophoresis, and by analysis of several recombinant clones. The studies have included both antibody-based and T cell stimulation assays. Bet v 1, the major **allergen** of birch, forms at least 24 spots when conventional extracts are analyzed by 2D gel electrophoresis. Comparison of Bet v 1-encoding DNA sequences reveals a considerable number of **amino acid substitutions.** This sequence variation can theoretically account for the number of spots observed in 2D gels. Whereas pools of serum from allergic individuals and monospecific antibodies raised in rabbits bind to most if not all spots in 2D gels, analyses of individual serum and/or murine monoclonal antibodies show individual patterns of reactivity with various subsets of spots. These observations point to a model in which **amino acid substitutions** induce local perturbations of the **allergen** surface, causing differences in epitope structure. Furthermore, analysis of pollen from individual **trees** shows that each **tree** produces individual subsets of Bet v 1 spots. When analyzed in stimulation assays, T cell clones also display differences in reactivity to different isoallergens. In conclusion, we have shown that Bet v 1 is heterogeneous, and that individual **trees** produce various subsets of isoallergens which display differences in reactivity both towards antibodies and T cells. A careful selection of isoform may therefore be

of major importance if recombinant **allergens** or synthetic peptides are to be used for conventional immunotherapy.

=> s l12 and weed
L37 0 L12 AND WEED

=> s l12 and weeds
L38 0 L12 AND WEEDS

=> s l12 and animal
L39 63 L12 AND ANIMAL

=> dup remove l39
PROCESSING COMPLETED FOR L39
L40 45 DUP REMOVE L39 (18 DUPLICATES REMOVED)

=> d l40 1-45 cbib abs

L40 ANSWER 1 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2004:209728 Document No.: PREV200400212348. Degenerate recognition and response of human CD4+ Th cell clones: Implications for basic and applied immunology. Nishimura, Yasuharu [Reprint Author]; Chen, Yu-Zhen; Uemura, Yasushi; Tanaka, Yoshihiko; Tsukamoto, Hirotake; Kanai, Takayuki; Yokomizo, Hiroshi; Yun, Chyuns; Matsuoka, Takako; Irie, Atsushi; Matsushita, Sho. Department of Immunogenetics, Graduate School of Medical Sciences, Kumamoto University, Honjo 1-1-1, Kumamoto, 860-8556, Japan. mxnishim@gpo.kumamoto-u.ac.jp. Molecular Immunology, (February 2004) Vol. 40, No. 14-15, pp. 1089-1094. print.
ISSN: 0161-5890 (ISSN print). Language: English.
AB It was once considered that the T cell response is an all or nothing type event, but recent studies have clearly indicated that T cells show many different types of activation in recognition of altered ligands for T cell receptors (TCR). In this review, we summarize our recent findings on the response of human CD4+ helper T (Th) cell clones to altered peptide ligands (APL); peptides carrying single or multiple residue substitutions in antigenic peptides. The extensive analyses revealed that TCR-antagonism and partial agonism are frequently observed by the stimulation with APLs substituted at particular amino acid residues of antigenic peptides. We observed unique partially agonistic APLs inducing prolongation of T cell survival without cell proliferation. Superagonistic APLs stimulated enhanced proliferation and production of cytokines in Th cell clones reactive to tumor-associated antigens. The other APL induced enhanced production of interleukin-12 by antigen presenting cells and subsequent enhancement of IFN-gamma production by T cells reactive to **allergens.** By utilizing an HLA-DR-restricted T cell epitope library generated by mutated invariant chain genes, it was revealed that human Th cell clones recognize a more diverse array of peptides with multiple and simultaneous **amino acid substitutions** in an antigenic peptide. APLs also induced altered intracellular signaling events including intracellular calcium increase and phosphorylation of signaling molecules. This information provides basic knowledge regarding the characteristics of antigen recognition by human Th cells and the subsequent activation, and a novel method for manipulation of human Th cell responses by APLs, as a possible candidate for antigen-specific immuno-potentiating or immunosuppressive therapy.

L40 ANSWER 2 OF 45 MEDLINE on STN DUPLICATE 1
2003420824. PubMed ID: 12960334. Dominating IgE-binding epitope of Bet v 1, the major **allergen** of birch pollen, characterized by X-ray crystallography and site-directed mutagenesis. Spangfort Michael D; Mirza Osman; Ipsen Henrik; Van Neerven R J Joost; Gajhede Michael; Larsen Jorgen N. (ALK-Abello, Research Department, Horsholm, Denmark.. msp@dk.alk.abello.com) . Journal of immunology (Baltimore, Md. : 1950), (2003 Sep 15) 171 (6) 3084-90. Journal code: 2985117R. ISSN: 0022-1767.

Pub. country: United States. Language: English.

AB Specific allergy vaccination is an efficient treatment for allergic disease; however, the development of safer vaccines would enable a more general use of the treatment. Determination of molecular structures of **allergens** and **allergen**-Ab complexes facilitates epitope mapping and enables a rational approach to the engineering of **allergen** molecules with reduced IgE binding. In this study, we describe the identification and modification of a human IgE-binding epitope based on the crystal structure of Bet v 1 in complex with the BV16 Fab' fragment. The epitope occupies approximately 10% of the molecular surface area of Bet v 1 and is clearly conformational. A synthetic peptide representing a sequential motif in the epitope (11 of 16 residues) did not inhibit the binding of mAb BV16 to Bet v 1, illustrating limitations in the use of peptides for B cell epitope characterization. The single **amino acid substitution**, Glu(45)-Ser, was introduced in the epitope and completely abolished the binding of mAb BV16 to the Bet v 1 mutant within a concentration range 1000-fold higher than wild type. The mutant also showed up to 50% reduction in the binding of human polyclonal IgE, demonstrating that glutamic acid 45 is a critical amino acid also in a major human IgE-binding epitope. By solving the three-dimensional crystal structure of the Bet v 1 Glu(45)-Ser mutant, it was shown that the change in immunochemical activity is directly related to the Glu(45)-Ser substitution and not to long-range structural alterations or collapse of the Bet v 1 mutant tertiary structure.

L40 ANSWER 3 OF 45 MEDLINE on STN

2003253972. PubMed ID: 12778490. The immunodominant epitope of lipocalin **allergen** Bos d 2 is suboptimal for human T cells. Kinnunen Tuure; Buhot Cecile; Narvanen Ale; Rytkonen-Nissinen Marja; Saarelainen Soili; Pouvelle-Moratille Sandra; Rautiainen Jaakko; Taivainen Antti; Maillere Bernard; Mantyjarvi Rauno; Virtanen Tuomas. (Department of Clinical Microbiology, University of Kuopio, Finland. ) European journal of immunology, (2003 Jun) 33 (6) 1717-26. Journal code: 1273201. ISSN: 0014-2980. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB We have proposed earlier that the poor capacity of the lipocalin **allergen** Bos d 2 to stimulate highly allergic subjects' peripheral blood mononuclear cells could be ascribed to endogenous lipocalins and could be related to the allergenic potential of the molecule. Here, we have characterized the proliferative and cytokine responses of human T cell clones against the immunodominant epitope of Bos d 2. We observed, for clone F1-9, that a substitution of aspartic acid for asparagine in the core region of the epitope increased the stimulatory capacity of the peptide about 100-fold in comparison with the natural peptide. For clone K3-2, from a different patient, the substitution of lysine for glutamine or isoleucine for leucine in the core region resulted in about 30-fold and 10-fold increases in the stimulatory capacity of the peptides, respectively. The clones also recognized self-protein-derived peptides but not the peptides derived from other lipocalins. We suggest that the poor recognition of the immunodominant epitope of Bos d 2 can be a factor accounting for Bos d 2-allergic subjects' weak cellular responses. Suboptimal recognition of self and **allergen** epitopes by T cells may be of significance for the allergenicity of proteins.

L40 ANSWER 4 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN

2002:946065 Document No. 138:38056 Mutant forms of cholera holotoxin as an adjuvant. Green, Bruce A.; Holmes, Randall K.; Jobling, Michael G.; Zhu, Duzhang (American Cyanamid Company, USA; Government of the United States of America as Represented by the Uniformed Services University of the Health Sciences). PCT Int. Appl. WO 2002098369 A2 20021212, 88 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO,

RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US21008 20020605. PRIORITY: US 2001-PV296531 20010607.

AB Mutant cholera holotoxins having single or double **amino acid substitutions** or insertions have reduced toxicity compared to the wild-type cholera holotoxin. The mutant cholera holotoxins are useful as adjuvants in antigenic compns. to enhance the immune response in a vertebrate host to a selected antigen from a pathogenic bacterium, virus, fungus, or parasite, a cancer cell, a tumor cell, an **allergen**, or a self-mol.

L40 ANSWER 5 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN

2002:946064 Document No. 138:23652 Mutant forms of cholera holotoxin as an adjuvant. Green, Bruce A.; Holmes, Randall K.; Jobling, Michael G.; Zhu, Duzhang (American Cyanamid Company, USA; University of Colorado). PCT Int. Appl. WO 2002098368 A2 20021212, 89 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US20978 20020605. PRIORITY: US 2001-PV296537 20010607.

AB Mutant cholera holotoxins comprising a cholera toxin subunit A having single **amino acid substitutions** in the amino acid positions (16 or 72) or double amino acid positions (16 and 68) or (68 and 72) have reduced toxicity compared to the wild-type cholera holotoxin. The mutant cholera holotoxins are useful as adjuvants in immunogenic compns. to enhance the immune response in a vertebrate host to a selected antigen from a pathogenic bacterium, virus, fungus, or parasite, a cancer cell, a tumor cell, an **allergen**, or a self-mol.

L40 ANSWER 6 OF 45 MEDLINE on STN

2003010201. PubMed ID: 12516563. Emerging principles for the design of promiscuous HLA-DR-restricted peptides: an example from the major bee venom **allergen.** Texier Catherine; Pouvelle-Moratille Sandra; Buhot Cecile; Castelli Florence A; Pecquet Catherine; Menez Andre; Leynadier Francisque; Maillere Bernard. (Protein Engineering and Research Department, CEA-Saclay, Gif sur Yvette, France. ) European journal of immunology, (2002 Dec) 32 (12) 3699-707. Journal code: 1273201. ISSN: 0014-2980. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB Mechanisms underlying successful immunotherapy of allergic patients operate at the level of CD4+ helper T cells. T cell epitopes from **allergens** may thus constitute interesting molecules for immunotherapy, provided they are efficient for all patients and are not recognized by IgE. In an attempt to define such peptides for allergy to bee venom, we have investigated the capacity of peptides encompassing the sequence of the major bee venom **allergen** to stimulate PBMC from allergic patients and to react specifically with their IgE. The region 77-110 emerged as the most frequently T cell stimulating. We then analyzed the binding modes of the sequence 81-97 for ten different HLA-DR molecules and introduced punctual mutations to enhance the peptide affinity for these molecules. Six different modes have been identified on the sequence 81-97, one mode being common to eight HLA-DR molecules. Four HLA-DR molecules can bind the P85-97 peptide by two different modes with an equivalent affinity. The peptide N89L has a higher affinity for DRB1*0301 and DRB3*0101 and remains as active as the native peptide towards the other HLA-DR molecules.

L40 ANSWER 7 OF 45 MEDLINE on STN DUPLICATE 2
2002436205. PubMed ID: 12193711. The Dermatophagoides pteronyssinus group 2 **allergen** contains a universally immunogenic T cell epitope. Wu Bo; Elst Luc Vander; Carlier Vincent; Jacquemin Marc G; Saint-Remy Jean-Marie R. (Center for Molecular and Vascular Biology, University of Leuven, Belgium. ) Journal of immunology (Baltimore, Md. : 1950), (2002 Sep 1) 169 (5) 2430-5. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB The use of T cell epitope-containing peptides for the induction of anergy in **allergen** sensitization is limited by genetic restriction that could be circumvented by using universally immunogenic epitopes. We attempted to identify such epitopes on Dermatophagoides pteronyssinus group 2 **allergen** (Der p 2), a major **allergen** of D. pteronyssinus T cells from BALB/c (H-2(d)), C57BL/6 (H-2(b)), C3H (H-2(k)), and SJL (H-2(s)) mice that were immunized with rDer p 2, recognized an immunodominant region encompassing residues 21-35. A synthetic 21-35 peptide (p21-35) induced strong dose-dependent in vitro T cell proliferation with cells of the four mouse strains and required processing for MHC class II presentation. Substitution of Ile(28) with Ala resulted in reduction of T cell proliferation in each strain. Ile(28) could represent an important MHC class II anchoring residue for T cell response to p21-35. An immunodominant T cell epitope of Der p 2 therefore behaves as a universal epitope and could be a suitable candidate for T cell anergy induction.

L40 ANSWER 8 OF 45 MEDLINE on STN
2002211600. PubMed ID: 11944924. Identification and fine mapping of IgG and IgE epitopes in ovomucoid. Mine Yoshinori; Wei Zhang Jie. (Department of Food Science, University of Guelph, Guelph, Ontario, N1G2W1, Canada.. ymine@uoguelph.ca) . Biochemical and biophysical research communications, (2002 Apr 12) 292 (4) 1070-4. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB Ovomucoid is a major **allergen** in hen egg white which causes a serious IgE-mediated food allergy reaction. This study determined eight IgG epitopes, 5-11 amino acids in length, and nine IgE epitopes, 5-16 amino acids in length, within the primary sequence in ovomucoid using arrays of overlapping peptides synthesized on cellulose membranes. Pooled sera from eight egg-allergic patients were used to probe the membrane. We also analyzed the amino acids that are critical for antibody binding by substituting a single amino acid within each epitope. Mutational analysis of the epitopes indicated that charged amino acids (aspartic acid, glutamic acid, and lysine) and some hydrophobic (leucine, phenylalanine, and glycine) and polar (serine, threonine, tyrosine, and cystein) amino acids were important for antibody binding. These results provide useful information for the molecular design necessary to reduce the allergenicity of ovomucoid, and a better understanding of structure-function relationships of allergic epitopes in food proteins.


L40 ANSWER 9 OF 45 MEDLINE on STN DUPLICATE 3
2002306171. PubMed ID: 12047443. Stimulatory and inhibitory epitopes in the T cell responses of mice to Der p 1. Jarnicki A G; Thomas W R. (Centre for Child Health Research, University of Western Australia, TVW Telethon Institute for Child health Research, West Perth, Western Australia. ) Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (2002 Jun) 32 (6) 942-50. Journal code: 8906443. ISSN: 0954-7894. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: The responses of mice to the mite **allergen** Der p 1 have been used to study the mechanisms of allergic sensitization and the development of new types of immunotherapy. Many of the studies require a knowledge of the T cell epitopes, and because Der p 1 is polymorphic, the effect of natural **amino acid substitution** in the **allergen.** The intranasal administration of peptides

containing T cell epitopes can induce a mucosal tolerance but it is not known if the major activity is limited to stimulatory peptides and if, as found for autoimmunity, some epitopes are not inhibitory. OBJECTIVE: To determine and compare the sequences of Der p 1 which contain stimulatory epitopes for the high responding H-2(b) and H-2(q) mice and the sequences which induce tolerance by intranasal administration of peptides. METHODS: T cell responses of mice immunized with Der p 1 were measured by in vitro T cell stimulation assays so an extensive study of epitope recognition and intranasal tolerance could be made. Synthetic peptides were used to examine the stimulatory and inhibitory ability of all Der p 1 sequences and to map the major H-2(b) epitope in detail. This included the effect of the common polymorphic amino acid 124 substitution found within this epitope. RESULTS: Three and two regions, respectively, were found to contain stimulatory T cell epitopes for H-2(b) and H-2(q) mice. The peptides in these regions were also the most active at inducing intranasal tolerance for the responding haplotype. The correspondence between inhibitory and stimulatory peptides was maintained for the fine mapping of the major H-2(b) epitope. This was found about a core region of 118-126 which was overlapping but separate to a consensus sequence for the binding of endogeneous peptides. Peptides with alanine at the naturally polymorphic residue 124 stimulated and inhibited responses to Der p 1 more effectively, while peptides with the valine 124 variant were immunogenic but poorly cross-reactive. CONCLUSIONS: The intranasal administration of peptides representing each of five epitopes recognized by two strains of mice were able to induce mucosal tolerance and the major tolerizing activity was limited to these epitopes. The position of the core major epitope for C57 mice, which differs from a previously predicted epitope, and its specificity for the natural alanine 124 variant is described.

L40 ANSWER 10 OF 45 MEDLINE on STN DUPLICATE 4
2002106797. PubMed ID: 11818327. Cockroach **allergen** Bla g 2: structure, function, and implications for allergic sensitization. Pomes Anna; Chapman Martin D; Vailes Lisa D; Blundell Tom L; Dhanaraj Venugopal. (Asthma and Allergic Diseases Center, Department of Internal Medicine, University of Virginia Health System, Charlottesville, VA, 22903, USA.. apomes@inbio.com) . American journal of respiratory and critical care medicine, (2002 Feb 1) 165 (3) 391-7. Journal code: 9421642. ISSN: 1073-449X. Pub. country: United States. Language: English.

AB Exposure to German cockroach (Blattella germanica) **allergens** is associated with the development of chronic respiratory diseases, especially asthma. The mechanism by which allergic patients develop specific immunoglobulin E (IgE) responses to environmental **allergens** is unknown. However, recent reports provided evidence that enzyme activity, especially proteolytic activity, was a major contributor to allergenicity. Bla g 2 is one of the most potent cockroach **allergens** (prevalence of IgE responses of 60 to 80%) and shows homology to the aspartic proteinase family of enzymes. We investigated whether the allergenicity of Bla g 2 was linked to its putative enzymatic function. A molecular model of Bla g 2, based on the high resolution crystal structures of pepsin and chymosin, showed that the overall three-dimensional structure of Bla g 2 was similar to that of aspartic proteinases with a well-defined binding pocket. However, critical **amino acid substitutions** in the catalytic triads and in the "flap" region of the molecule suggested that Bla g 2 was inactive and homologous to mammalian pregnancy-associated glycoproteins. This was confirmed experimentally by enzyme assay. The results show dissociation between enzymatic activity and allergenicity for Bla g 2 and suggest that other genetic and environmental factors are important determinants of sensitization.

L40 ANSWER 11 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2002:153564 Document No.: PREV200200153564. Linear IgE epitope mapping of the English walnut (Juglans regia) major food **allergen**, Jug r 1. Robotham, Jason M.; Teuber, Suzanne S.; Sathe, Shridhar K.; Roux, Kenneth H. [Reprint author]. Department of Biological Science, Biology Unit I,

Florida State University, Tallahassee, FL, 32306-4370, USA. Journal of Allergy and Clinical Immunology, (January, 2002) Vol. 109, No. 1, pp. 143-149. print.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

AB Background: Peanut and tree nut allergies can be life-threatening, and they appear to be growing in prevalence. Jug r 1, a 2S albumin seed storage protein, was previously characterized as a major English walnut food **allergen.** Objective: We sought to identify the linear IgE-binding epitopes of Jug r 1 and to determine which, if any, amino acids are necessary for this binding to occur. Methods: Pools of sera from walnut-allergic patients and overlapping peptides synthesized on an activated cellulose membrane were used to screen for IgE-binding epitopes. Mutational analysis of the immunodominant epitope was carried out through single and multisite **amino acid substitutions** . Inhibition assays were performed through use of affinity-purified IgE, soluble forms of the epitope peptide, and the recombinant 2S albumin, rJug r 1. Results: One immunodominant linear epitope was identified. Amino acid mutations to the epitope demonstrated that the residues RGEE, at positions 36 through 39, were minimally required for IgE binding. Probing of this epitope with sera from each of 20 patients revealed 15 of the sera to be positive. Binding of patients' IgE to the epitope was inhibited with a soluble form of the peptide; however, soluble peptide did not completely inhibit the binding of IgE to the intact rJug r 1. Conclusion: One major linear IgE-reactive epitope and its critical core amino acid residues have been identified. Mutation of any of these core amino acids resulted in loss of IgE binding to the epitope, and this points toward the feasibility of reducing allergenicity in genetically modified walnuts. However, strong evidence for the existence of conformational epitopes was also obtained.

L40 ANSWER 12 OF 45 MEDLINE on STN DUPLICATE 5

2002322196. PubMed ID: 12023195. Current understanding of food **allergens.** Lehrer Samuel B; Ayuso Rosalia; Reese Gerald. (Section of Clinical Immunology, Allergy and Rheumatology, Department of Medicine, Tulane University School of Medicine, New Orleans, Louisiana 70112, USA.. sblehrer@tulane.edu) . Annals of the New York Academy of Sciences, (2002 May) 964 69-85. Ref: 39. Journal code: 7506858. ISSN: 0077-8923. Pub. country: United States. Language: English.

AB Food allergies are IgE-mediated immunological reactions; this distinguishes them from other adverse reactions to foods. Most (>90%) of the recognized food allergies are generally thought to be caused by eight foods or food groups. A number of factors can affect food allergy development, including diet and culture, route of exposure, processing, cooking, and digestion. In addition, it is thought that the properties of certain food proteins render them more likely to be allergenic than other proteins. Most food **allergens** are major proteins, polyvalent molecules with at least two or more IgE-binding sites, and are recognized as foreign molecules (hence immunogenic). A number of major food **allergens** have been recently characterized, and amino acid sequences determined. Tropomyosin is the only major **allergen** of shrimp. A number of IgE-binding epitopes have been identified in this molecule, though they may vary from one shrimp-allergic individual to another. Single **amino acid substitutions** within epitopes based on that of homologous, nonreactive tropomyosins can substantially enhance or abolish IgE antibody binding. Using the accumulated knowledge of food **allergen** protein structure, the allergenicity of novel proteins to which there has been no prior human exposure has been assessed. This has been based primarily on the lability or resistance of a protein to enzymatic degradation. Clearly, further criteria must be developed to refine this process. In this regard, the development of **animal** models that have been sufficiently validated as surrogates of human IgE antibody responses is needed for more precise assessment of the allergenic potential of proteins.

L40 ANSWER 13 OF 45 MEDLINE on STN DUPLICATE 6

2002615868. PubMed ID: 12372997. Molecular basis of arthropod cross-reactivity: IgE-binding cross-reactive epitopes of shrimp, house dust mite and cockroach tropomyosins. Ayuso Rosalia; Reese Gerald; Leong-Kee Susan; Plante Matthew; Lehrer Samuel B. (Section of Allergy and Clinical Immunology, Tulane University School of Medicine, 1700 Perdido Street, New Orleans, LA 70112, USA. ) International archives of allergy and immunology, (2002 Sep) 129 (1) 38-48. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Shrimp may cross-react with other crustaceans and mollusks and nonedible arthropods such as insects (cockroach and chironomids), arachnids (house dust mites) and even nematodes. Since the muscle protein tropomyosin has been implicated as a possible cross-reacting **allergen**, this study characterized the IgE-binding epitopes in shrimp tropomyosin, Pen a 1, that cross-react with other allergenic invertebrate tropomyosins in house dust mites (Der p 10, Der f 10) and cockroaches (Per a 7). Pen a 1-reactive sera from shrimp-allergic subjects were used to evaluate the effect on IgE binding of different **amino acid substitutions** in Pen a 1 epitopes based on homologous sequences in Per a 7 and Der p 10/Der f 10. METHODS: Peptides were synthesized spanning the length of Pen a 1 IgE-binding epitopes and **amino acid substitutions** were performed based on homologous amino acid sequences from Per a 7 and Der p 10/Der f 10. RESULTS: 7/8 individually recognized Pen a 1 epitopes (2, 3a, 3b, 4, 5a, 5b and 5c) had an identical amino acid sequence with lobster **allergen**, Hom a 1, 4/8 (3a, 3b, 4 and 5a) with Der p 10 and Der f 10, and 5/8 (2, 3a, 3b, 4 and 5a) with Per a 7. In addition, even homologous regions of other arthropod tropomyosins that differ in one or more amino acids from the sequences of Pen a 1 epitopes are still recognized by shrimp-allergic IgE antibodies. In total, shrimp-allergic sera recognize 6/8 peptides homologous to Pen a 1 epitopes in Per a 7, 7/8 in Der p 10/Der f 10, and 7/8 epitopes in Hom a 1. CONCLUSIONS: The IgE recognition by shrimp-allergic individuals of identified and/or similar amino acid sequences homologous to Pen a 1 epitopes in mite, cockroach and lobster tropomyosins are the basis of the in vitro cross-reactivity among invertebrate species. Based on amino acid sequence similarity and epitope reactivity, lobster tropomyosin has the strongest and cockroach the least cross-reactivity with shrimp. The clinical relevance of these cross-reactivities in developing allergic reactions to different arthropods needs to be determined.



L40 ANSWER 14 OF 45 MEDLINE on STN DUPLICATE 7

2001350468. PubMed ID: 11398074. The molecular basis of antigenic cross-reactivity between the group 2 mite **allergens.** Smith A M; Benjamin D C; Hozic N; Derewenda U; Smith W A; Thomas W R; Gafvelin G; van Hage-Hamsten M; Chapman M D. (Asthma & Allergic Diseases Center, Department of Medicine, University of Virginia Health System, Charlottesville, VA 22908-1355, USA. ) Journal of allergy and clinical immunology, (2001 Jun) 107 (6) 977-84. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Mite group 2 **allergens** Der p 2, Der f 2, and Eur m 2 are 14-kDa proteins of unknown function that share 83% to 85% amino acid sequence identity. Isoforms of the **allergens** within each genus have been identified which differ by 3 or 4 amino acids, but little is known of the influence of group 2 polymorphisms on human IgE antibody binding. OBJECTIVE: The purpose of this study was to investigate the importance of interspecies and isoform substitutions on murine mAb and IgE antibody binding and on the molecular structure of the group 2 **allergens.** METHODS: Site-directed mutagenesis was used to incorporate the isoform **amino acid substitutions** onto the Der p 2.0101 sequence. Recombinant **allergens** were expressed and purified from Escherichia coli and used to evaluate antibody binding by enzyme-linked immunosorbent assay (ELISA). Molecular modeling of the tertiary structure was used to analyze structural differences between the various group 2 **allergens.**

RESULTS: The substitution of asparagine for aspartic acid at position 114 restored mAb binding of rDer p 2.0101; the other Der p 2 isoforms and the 3 rDer f 2 isoforms also reacted in the 2-site ELISA. The correlation of IgE binding to the Der p 2 isoforms was excellent and tended to be higher in the isoforms with the asparagine 114 substitution (r (2) = 0.87 vs r (2) = 0.95). rEur m 2.0101 bound to all mAb except 7A1; when compared with rDer p 2 for IgE binding, rEur m 2.0101 gave a correlation coefficient of r (2) = 0.68. Molecular modeling revealed that Eur m 2 and the storage mite homologs Lep d 2 and Tyr p 2 retain the tertiary fold of Der p 2. Eur m 2 has a conserved surface, whereas Lep d 2 and Tyr p 2 present most of the **amino acid substitutions** on this surface. Lep d 2 and Tyr p 2 did not react with mAb or with sera from patients with IgE to Dermatophagoides species. CONCLUSION: The isoform substitutions of rDer p 2 can be distinguished by mAb. The allergenic cross-reactivity between Der p 2, Der f 2, and Eur m 2 is a direct result of the conserved antigenic surface, whereas the lack of cross-reactivity with Lep d 2 and Tyr p 2 is a result of the multiple substitutions across this surface.

L40 ANSWER 15 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2001:327462 Document No.: PREV200100327462. Molecular basis of allergic cross-reactivity between group 1 major **allergens** from birch and apple. Holm, J.; Baerentzen, G.; Gajhede, M.; Ipsen, H.; Larsen, J. N.; Lowenstein, H.; Wissenbach, M.; Spangfort, M. D. [Reprint author]. Biochemical Allergy Research, ALK-Abello A/S, Boge Alle 6-8, DK-2970, Horsholm, Denmark. msp@dk.alk-abello.com. Journal of Chromatography B, (25 May, 2001) Vol. 756, No. 1-2, pp. 307-313. print.
CODEN: JCBADL. ISSN: 0378-4347. Language: English.

AB Patients allergic to birch pollen often also react with fruits and vegetables, such as apple. The major cause of cross-reactivity between birch and apple is biochemical and immunological similarity between the major **allergens**, Bet v 1 and Mal d 1, as demonstrated by serological and cellular immunoassays. In addition, birch pollen-specific therapeutic allergy vaccination has been shown to improve allergic symptoms caused by oral ingestion of apple. Detailed analysis of molecular surface areas based on the crystal structure of Bet v 1, and primary sequence alignment, identify potential epitopes for cross-reactive antibodies. Two or more conserved patches are identified when comparing Bet v 1 and Mal d 1, thus providing a molecular model for serological cross-reactivity involving more than one IgE-binding epitope. A minimum of two epitopes would be necessary for cross-linking of receptor bound IgE in functional histamine release assays and skin test. Individual **amino acid substitutions**, as occurring in isoallergenic variation, may, however, have a dramatic effect on epitope integrity if critical residues are affected. Thus, one area large enough to accommodate antibody-binding epitopes shared by all known Mal d 1 isoallergens and variants is identified, as well as areas shared by Bet v 1 and individual Mal d 1 isoallergens or variants. The occurrence of limited epitope coincidence between Bet v 1 and Mal d 1 is in agreement with the observation that some, but not all, birch pollen allergic patients react with apple, and that the epitope repertoire recognised by the IgE of the individual patients determines the degree of cross-reactivity.

L40 ANSWER 16 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2001:507136 Document No.: PREV200100507136. Effect of **amino acid substitutions** on the IgE binding capacity of major epitopes of the shrimp **allergen** Pen a 1 (tropomyosin): Implications for the development of a hypoallergenic isoform for immunotherapy. Reese, G. [Reprint author]; Ayuso, R. [Reprint author]; Lehrer, S. B. [Reprint author]. Medicine/Clinical Immunology, Tulane University Health Sciences Center, New Orleans, LA, USA. Allergy (Copenhagen), (2001) Vol. 56, No. Supplement 68, pp. 269. print.
Meeting Info.: XXth Congress of the European Academy of Allergology and Clinical Immunology. Berlin, Germany. May 09-13, 2001.

CODEN: LLRGDY. ISSN: 0105-4538. Language: English.

L40 ANSWER 17 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN
2001:619933 Document No. 135:327409 Immunomodulation by the submandibular gland. Forsythe, Paul; Dery, Rene E.; Mathison, Ronald; Davison, Joseph S.; Befus, A. Dean (Pulmonary Research Group, Department of Medicine, Faculty of Medicine, University of Alberta, Edmonton, AB, T6G 2S2, Can.). Neuroimmune Biology, 1(New Foundation of Biology), 203-224 (English) 2001. CODEN: NBEIAQ. ISSN: 1567-7443. Publisher: Elsevier Science B.V..

AB A review with refs. The authors have established that decentralization (cutting sympathetic nerve trunk) of the superior cervical ganglia bilaterally reduces the magnitude of allergic inflammation in the airways of rats. The magnitude of anaphylactic and endotoxic hypotension, and of gastrointestinal inflammation was also reduced. This anti-inflammatory activity was dependent upon intact submandibular glands. Reconstitution of sialadenectomized (removal of submandibular glands) rats with soluble exts. of the submandibular glands identified two polypeptides with anti-inflammatory activities. The sequences of these polypeptides were found within a prohormone, submandibular gland rat 1 (SMR1). The C-terminal peptide TDIFEGG, has been studied extensively. Using sequential **amino acid substitutions** and systematic removal of C terminal or N terminal amino acids, the authors established that the tripeptide FEG is biol. active. Modification of FEG to the D-isomeric feG, enhances its activity in some assay systems. The authors postulated that feG would inhibit airways inflammation, and tested this using a model of allergic asthma, namely the Brown Norway rat sensitized to ovalbumin (OA). Sensitized rats were challenged 14 to 21 days later with aerosolized OA. This challenge markedly increased nos. of inflammatory cells recovered from the airways after 24 h (29 + 106, n = 23) compared to saline controls (1 + 106, n = 4). The infiltrating cells included macrophages (10 + 106), neutrophils (9 + 106) and eosinophils (9 + 106). I.v. (0.25 mg/kg) or oral feG (1 mg/kg) given 30 min prior to OA significantly inhibited influx of inflammatory cells by 50 to 70%. FeG reduced inflammatory cell infiltration when given 30 min before to 3 to 6 h post **allergen** exposure. Oral feG reduced the nos. of macrophages, neutrophils and eosinophils. One of the mechanisms underlying the effects of feG may be its ability to inhibit PAF-induced expression of CD11b on purified human neutrophils. It is possible that feG may be useful in the treatment of allergic asthma, given either as an oral prophylactic, or as a post exposure therapeutic. The cervical sympathetic nerve trunk-submandibular gland axis of neuroendocrine regulation of inflammation may be dysfunctional in inflammatory diseases and provide opportunities for new therapeutic intervention. This axis may be sensitive to modulation by central and peripheral neural mechanisms that influence its function/dysfunction.

L40 ANSWER 18 OF 45 MEDLINE on STN
2002084678. PubMed ID: 11811644. The effect of fungal ribosome inactivating proteins upon feeding choice in C. freemani, and indications of a mutualistic relationship with A. restrictus. Environmental mycology. Brandhorst T; Dowd P F; Kenealy W R. (Department of Pediatrics, University of Wisconsin Medical School, University of Wisconsin Hospital and Clinics, Madison 53792, USA.. Tbrandho@facstaff.wisc.edu) . Mycopathologia, (2001) 152 (3) 155-8. Journal code: 7505689. ISSN: 0301-486X. Pub. country: Netherlands. Language: English.

AB Carpophilus freemani beetles' feeding on the fungus Aspergillus nidulans was substantially inhibited when A. nidulans was transformed and induced to secrete the ribosome inactivating protein, restrictocin (genetic source: Aspergillus restrictus). No inhibition of feeding was observed when A. nidulans was transformed and induced to produce an inactive form of restrictocin with a single **amino-acid substitution** in the active site. Similarly, there was no inhibition of feeding upon transgenic strains when the production of restrictocin was not induced. Feeding inhibition of C. freemani by

restrictocin requires that the ribonuclease be active and is not due to other characteristics of the protein or the transgenic host fungus.

L40 ANSWER 19 OF 45 MEDLINE on STN
2001209860. PubMed ID: 11298012. How to make foods safer--genetically modified foods. Moseley B E. (Reading, Berkshire, UK. ) Allergy, (2001) 56 Suppl 67 61-3. Ref: 7. Journal code: 7804028. ISSN: 0105-4538. Pub. country: Denmark. Language: English.
AB It is the responsibility of companies developing genetically modified foods, and of regulatory authorities that approve their marketing, to ensure that they are at least as safe as the traditional foods they are intended to replace in the diet. This requires that any novel material introduced into the food material should not be allergenic. If the novel gene has come from an allergenic source, e.g. nuts, it is necessary to demonstrate using immunological procedures applied to the IgE fractions of pooled sera from individuals with confirmed allergies that the novel protein is non-allergenic. When the novel gene is from a non-allergenic source then it is necessary to demonstrate lack of significant amino acid sequence homology to known **allergens** together with sensitivity to food manufacturing and digestive processes. Consumer confidence in genetically modified foods would be significantly improved if hypoallergenic varieties of crops and food products that are currently allergenic could be developed. Techniques such as antisense technology and single site **amino acid substitution** have been shown to have such potential.

L40 ANSWER 20 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN
2001:84448 Document No. 134:99748 The study on characteristics of the gut-associated immune system concerning generation and suppression of food allergy. Kaminogawa, Shuichi (Dep. Appl. Biol. Chem., The Univ. Tokyo, Japan). Nippon Nogei Kagaku Kaishi, 75(1), 1-20 (Japanese) 2001. CODEN: NNKKAA. ISSN: 0002-1407. Publisher: Nippon Nogei Kagakkai.
AB A review with 95 refs., on the mol. mechanism of food allergy, mechanism of the recognition of **allergens** (αs1-casein and β-lactoglobulin) by T cell and B cell, antigen-specific inhibition of T cell responses to β-lactoglobulin by **amino acid substitution**, induction of TCR antagonism, **animal** model for food allergy, anal. of denaturation of **allergens** by monoclonal antibodies, roles of intestinal intraepithelial T lymphocytes (IEL) and Peyer's patch in gut-associated lymphoid tissue, differentiation and function of IEL, mol. mechanism of oral immune tolerance, application of oral immune tolerance in the treatment of allergy and autoimmune diseases, and suppression of allergic responses by food components (lactic acid bacteria, etc.).

L40 ANSWER 21 OF 45 MEDLINE on STN
2001061112. PubMed ID: 11054118. Effects of proline mutations in the major house dust mite **allergen** Der f 2 on IgE-binding and histamine-releasing activity. Takai T; Ichikawa S; Hatanaka H; Inagaki F; Okumura Y. (Bioscience Research and Development Laboratory, Asahi Breweries, Ltd, Ibaraki, Japan.. toshiro.takai@asahibeer.co.jp) . European journal of biochemistry / FEBS, (2000 Nov) 267 (22) 6650-6. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.
AB Der f 2 is the major group 2 **allergen** from house dust mite Dermatophagoides farinae and is composed of 129 amino-acid residues. Wild-type and six proline mutants of Der f 2 (P26A, P34A, P66A, P79A, P95A, and P99A) expressed in Escherichia coli were refolded and purified. Formations of intramolecular disulfide bonds in the purified proteins were confirmed correct. The apparent molecular masses analyzed by gel-filtration were 14-15 kDa. The IgE-binding capacity in the sera of seven mite-allergic patients, inhibitory activity for IgE-binding to immobilized wild-type Der f 2, and activity to stimulate peripheral blood basophils to release histamine in two volunteers were analyzed. P95A and P99A, which slightly differed from the wild-type Der f 2 in their CD

spectrum, showed reduced IgE-binding, reduced inhibitory activity, and less histamine-releasing activity than the wild-type. P34A also showed reduced allergenicity. Considering that Pro95, Pro99 and Pro34 are closely located in loops at one end of the tertiary structure of Der f 2, we concluded that these loop regions included an IgE-binding site common to all tested patients. P66A showed reduced IgE-binding in two sera out of seven. P26A and P79A showed no reduced allergenicity. However, in immunoblot analysis after SDS/PAGE under reduced conditions, P79A showed no or markedly reduced IgE-binding while the other mutants showed IgE-binding corresponding to that in the assay using correctly refolded proteins. This suggests that Pro79 is involved in refolding of Der f 2. The findings in this study are important for the understanding of the antigenic structure of mite group 2 **allergens** and for manipulation of the **allergens** for specific immunotherapy.

L40 ANSWER 22 OF 45 MEDLINE on STN

2000281698. PubMed ID: 10820288. Threshold signaling of human Th0 cells in activation and anergy: modulation of effector function by altered TCR ligand. Verhoef A; Lamb J R. (Department of Biology, Imperial College of Science, Technology and Medicine, London, United Kingdom. ) Journal of immunology (Baltimore, Md. : 1950), (2000 Jun 1) 164 (11) 6034-40. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Molecular interactions between TCR and its natural ligand, in the presence of costimulatory signals, elicit T cell effector functions, whereas subtle changes in the structure of antigenic peptides may induce only selected T cell effector function including anergy. In this study, we have investigated the immunological activity of an altered TCR ligand (p 2, 28-40A34,36) derived from the immunodominant T cell epitope of the group 2 **allergen** of house dust mite, in which residues at positions 34 and 36 were substituted by alanine. Elevated IFN-gamma synthesis was induced by equimolar concentrations of the analogue compared with native peptide (p 2, 28-40) and was paralleled by increased down-regulation of cell surface CD3. IL-5 and IL-10 production exhibit the same sensitivity to both peptides, implying that the induction of T cell effector functions are not all proportional to TCR occupancy. Both native peptide and the analogue bound to MHC class II (DRB1*1101) molecules with similar affinities. Furthermore, p 2, 28-40A34,36 induced T cell anergy at lower concentrations than native peptide. During the induction of anergy, TGF-beta production was comparable for both peptides, whereas IL-10 secretion was markedly increased but more so in response to p 2, 28-40A34,36. Membrane expression of costimulatory ligands CD80 and CD86 was similar for native peptide and p 2, 28-40A34,36 and increased in activation, whereas only CD86 was elevated during anergy. The modulation of T cell effector function with altered TCR ligands may have practical applications in reprogramming allergic inflammatory responses through the induction of T cell anergy and/or the promotion of Th1 cytokines.

L40 ANSWER 23 OF 45 MEDLINE on STN

2000437841. PubMed ID: 10946323. C8/119S mutation of major mite **allergen** Derf-2 leads to degenerate secondary structure and molecular polymerization and induces potent and exclusive Th1 cell differentiation. Korematsu S; Tanaka Y; Hosoi S; Koyanagi S; Yokota T; Mikami B; Minato N. (Department of Immunology and Cell Biology, Graduate School of Medicine, and Research Institute for Food Science, Kyoto University, Kyoto, Japan. ) Journal of immunology (Baltimore, Md. : 1950), (2000 Sep 1) 165 (5) 2895-902. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Hyposensitization therapy for atopic diseases has been conducted for decades but suffered from many problems including anaphylactic reactions. We previously developed a mutant protein of the major mite **allergen** Derf-2, C8/119S, which showed reduced binding to IgE. The C8/119S mutant was shown to exhibit more efficient hyposensitizing effect than Derf-2 in the **animal** model of allergic bronchial asthma. In the present study, we indicate that C8/119S exhibits markedly

augmented immunogenicity for the proliferation of Derf-2-specific human T cells and T cell clones irrespective of the epitope specificity as compared with Derf-2. Furthermore, C8/119S has induced potent and almost exclusive differentiation of Th1 cells from the peripheral blood of atopic patients in vitro. Neither Ag dosage effect nor absence of B cell-mediated Ag presentation could fully account for these effects. C8/119S has been indicated to lose the characteristic beta-barrel structure as judged by circular dichroism spectroscopic analysis and to polymerize solubly in physiological condition. Heating of Derf-2 also caused less stable molecular aggregation, but it hardly affected the secondary structure and failed to induce such a polarity toward the Th1 cell differentiation. These results have indicated that the degenerate secondary structure of C8/119S leading to stable molecular polymerization is primarily responsible for the marked increase in T cell-immunogenicity and the induction of exclusive Th1 cell differentiation in atopic patients. It has been suggested strongly that the recombinant C8/119S protein can provide an effective Ag with the least risk of anaphylaxis for **allergen** immunotherapy against house dust mite in human.

L40 ANSWER 24 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2000:130135 Document No.: PREV200000130135. Mutational analysis of the IgE-binding epitopes of P34/Gly m Bd 30K. Helm, Ricki M. [Reprint author]; Cockrell, Gael; Connaughton, Cathie; West, C. Michael; Herman, Eliot; Sampson, Hugh A.; Bannon, Gary A.; Burks, A. Wesley. Department of Pediatrics, Arkansas Children's Nutrition Center, University of Arkansas for Medical Sciences, 1120 Marshall St, Little Rock, AR, 72202, USA. Journal of Allergy and Clinical Immunology, (Feb., 2000) Vol. 105, No. 2 part 1, pp. 378-384. print.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.
AB Background: Peanuts and soybeans are 2 foods that have been shown to be responsible for many atopic disorders. Because of their nutritional benefit, soybean proteins are now being used increasingly in a number of food products. Previous studies have documented multiple **allergens** in soybean extracts, including glycinin, beta-conglycinin, and the P34/Gly m Bd 30K protein. Objective: Our overall goal was to identify soybean-specific **allergens** to begin to understand molecular and immunochemical characteristics of legume proteins. The specific aim of the current investigation was to identify the essential amino acid residues necessary for IgE binding in the 5 distinct immunodominant epitopes of P34/Gly m Bd 30K. Methods: Serum IgE from 6 clinically sensitive soybean-allergic individuals was used to identify P34/Gly m Bd 30K in the native and single amino acid substituted peptides with use of the SPOTS peptide synthesis technique to determine critical amino acids required for IgE binding. Results: The intensity of IgE binding and epitope recognition by serum IgE from the individuals varied substantially. With use of serum from 6 clinically soybean-sensitive individuals, 2 of the 5 immunodominant epitopes could be mutagenized to non-IgE binding peptides. Conclusions: Single-site **amino acid substitution** of the 5 immunodominant epitopes of Gly m Bd 30K with alanine revealed that IgE binding could be reduced or eliminated in epitopes 6 and 16 in the serum obtained from 6 soybean-sensitive patients.

L40 ANSWER 25 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 8
2001:160139 Document No.: PREV200100160139. Lack of an association of the Glu237Gly polymorphism in the gene for the Fcepsilon-receptor beta-subunit with atopic diseases in a Czech population. Schuller, M. [Reprint author]; Stelcl, M. [Reprint author]; Izakovicova-Holla, L. [Reprint author]; Vasku, A. [Reprint author]. Department of Pathological Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic. Scripta Medica (Brno), (2000) Vol. 73, No. 4, pp. 237-244. print.
CODEN: SCMEBF. ISSN: 0036-9721. Language: English.
AB The high-affinity IgE receptor (FcepsilonRI) plays a central role in degranulation of mast cells and basophils. This case-control study

investigated a possible association of the Glu237Gly polymorphism in exon 7 of the gene for the beta-subunit of this receptor with a predisposition to atopy in the Czech population. It included 157 patients (75 men and 82 women, aged 31+-15 years) with a history of one or more atopic conditions, i.e., asthma, allergic rhinitis or atopic dermatitis, and 77 healthy controls (40 men and 37 women, aged 40+-15 years). The Glu237Gly polymorphism was detected by means of PCR with a subsequent restriction analysis using the XmnI enzyme. Out of 234 subjects examined, only one patient was found to display an **amino acid substitution** (glutamic acid replaced by glycine) at position 237, which gave rise to a heterozygous combination of Gly237/Glu237. All the other subjects were homozygotes with a Glu237/Glu237 combination. Therefore, there was no reason for evaluating the statistical significance of differences between the patient and the control group. Our results clearly demonstrate that this mutation cannot be considered to be a polymorphism in our population. In some populations, this variant occurs more frequently and is associated with atopy, bronchial hyper-responsiveness, or a clinical manifestation of atopic asthma. However, the majority of recent studies, in agrreement with our results, have argued against an association of the Glu237Gly polymorphism with the development of atopy or asthma. These differences may be related to the effect of different environmental factor, particularly to the involvement of various **allergens** and parasitic infections which induce IgE-mediated immune reactions.

L40 ANSWER 26 OF 45 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2000405250 EMBASE Immune response of HLA-DQ transgenic mice to house dust mite **allergen** p2: Identification of HLA-DQ restricted minimal epitopes and critical residues. Krco C.J.; Harders J.; Chapoval S.; David C.S.. C.S. David, Department of Immunology, Mayo Clinic, Rochester, MN 55905, United States. david.chella@mayo.edu. Clinical Immunology 97/2 (154-161) 2000.
Refs: 34.
ISSN: 1521-6616. CODEN: CLIIFY. Pub. Country: United States. Language: English. Summary Language: English.

AB HLA-DQ8 (HLA-DQA1*0301; HLA-DQB1*0302) and HLA-DQ6 (HLA-DQA1*0103; HLA-DQB1*0602) genes were introduced into mouse class II (H-2A(β/°)), knockout mice. Transgenic HLA-DQ8 and HLA-DQ6 mice were individually immunized and challenged using synthetic peptides representing HDM (Dermatophagoides pteronyssinus) **allergen** p2. HLA-DQ8 mice responded to p2 peptides 1-20, 41-60, 51-70, 61-80, 91-110, and 101-120. HLA-DQ6 mice responded to peptides 1-20, 11-30, 21-40, 41-60, and 51-70. Using single amino acid truncated 30-mer peptides, residues necessary for HLA-DQ8 recognition were identified spanning regions 3-12, 50-70, and 91-120. A synthetic peptide comprising residues 3-12 was synthesized and a series of single alanine substitutions was introduced into the minimal peptide. Introduction of alanine residues at positions 3, 11, and 12 resulted in a significant loss of immune recognition. It was concluded that residues 4, 5, 7, 11, and 12 are critical for immune recognition by HLA-DQ8 mice. (C) 2000 Academic Press.

L40 ANSWER 27 OF 45 MEDLINE on STN DUPLICATE 9

2001054685. PubMed ID: 10919509. Use of altered peptide ligands to modulate immune responses as a possible immunotherapy for allergies. De Palma R; Sacerdoti G; Abbate G F; Martucci P; Mazzarella G. (Dipartimento di Internistica Clinica e Sperimentale F. Magrassi, Seconda Universita di Napoli, Naples, Italy.. rdepalma@netscape.net) . Allergy, (2000) 55 Suppl 61 56-9. Ref: 34. Journal code: 7804028. ISSN: 0105-4538. Pub. country: Denmark. Language: English.

AB Allergies are dramatically increasing in prevalence, and the management of these diseases is a heavy burden on the health-care systems of developed countries. In recent years, many efforts have been made to improve the therapy of allergies and to develop new approaches for immunotherapy. Here we briefly review the use of peptides to modulate T-cell responses to

**allergens.** We focus mainly on the possibility of using altered peptide ligands (APLs), i.e., peptides tailored on immunodominant T epitopes and bearing a single **amino-acid substitution**, as a tool to modulate immune responses to **allergens.** These peptides may be recognized by the specific T cells triggered by the agonist peptides, but they are unable to elicit T-cell responses; thus, they could be ideal candidates to modulate immune responses to **allergens.** The availability of these peptides could allow new approaches for immunotherapies.

L40 ANSWER 28 OF 45 MEDLINE on STN
1999364982. PubMed ID: 10433712. Role of individual cysteine residues and disulfide bonds in the structure and function of Aspergillus ribonucleolytic toxin restrictocin. Nayak S K; Rathore D; Batra J K. (Immunochemistry Laboratory, National Institute of Immunology, New Delhi, India. ) Biochemistry, (1999 Aug 3) 38 (31) 10052-8. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.
AB Restrictocin, produced by the fungus Aspergillus restrictus, belongs to the group of ribonucleolytic toxins called ribotoxins. It specifically cleaves a single phosphodiester bond in a conserved stem and loop structure in the 28S rRNA of large ribosomal subunit and potently inhibits eukaryotic protein synthesis. Restrictocin contains 149 amino acid residues and includes four cysteines at positions 5, 75, 131, and 147. These cysteine residues are involved in the formation of two disulfide bonds, one between Cys 5 and Cys 147 and another between Cys 75 and Cys 131. In the current study, all four cysteine residues were changed to alanine individually and in different combinations by site-directed mutagenesis so as to remove one or both the disulfides. The mutants were expressed and purified from Escherichia coli. Removal of any cysteine or any one of the disulfide bonds individually did not affect the ability of the toxin to specifically cleave the 28S rRNA or to inhibit protein synthesis in vitro. However, the toxin without both disulfide bonds completely lost both ribonucleolytic and protein synthesis inhibition activities. The active mutants, containing only one disulfide bond, exhibited relatively high susceptibility to trypsin digestion. Thus, none of the four cysteine residues is directly involved in restrictocin catalysis; however, the presence of any one of the two disulfide bonds is absolutely essential and sufficient to maintain the enzymatically active conformation of restrictocin. For maintenance of the unique stability displayed by the native toxin, both disulfide bonds are required.

L40 ANSWER 29 OF 45 MEDLINE on STN
1999254832. PubMed ID: 10323253. Association of polymorphisms in the beta2-adrenoreceptor gene with higher levels of parasitic infection. Ramsay C E; Hayden C M; Tiller K J; Burton P R; Hagel I; Palenque M; Lynch N R; Goldblatt J; LeSouef P N. (Department of Paediatrics, University of Western Australia, Perth, Australia. ) Human genetics, (1999 Mar) 104 (3) 269-74. Journal code: 7613873. ISSN: 0340-6717. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.
AB The diminishing incidence of parasitic infection in westernised societies has been suggested to result in an increased prevalance of asthma. Asthma is a polygenic disease and genome screens have shown that genes on chromosome 5q31-33 are strongly linked to the disease. The gene for the beta2-adrenoreceptor is located in this region and two polymorphisms have been identified that result in amino acid changes at positions 16 (ArgGly) and 27 (GlnGlu). To determine whether these polymorphisms influence asthma and parasitic infection, a genotype/phenotype study has been performed on a cohort of 126 children from Coche Island in Venezuela. There is a high incidence of asthma on the island and intestinal helminthiasis is endemic. Genotyping for both polymorphisms was carried out by using the polymerase chain reaction and allele-specific oligonucleotide hybridisation. Genotype frequencies in this cohort were consistent with other studies and both polymorphisms were in significant linkage disequilibrium. Individuals who were homozygous for Arg16 had significantly higher levels of specific IgE to Ascaris lumbricoides

(P=0.002), significantly higher A. lumbricoides egg counts (P<0.001) and significantly larger wheal sizes following skin-prick testing with A. lumbricoides **allergen** (P=0.008). There was no association between either polymorphism and total serum IgE or asthma in this population. A combination of mast cell degranulation and the lung migratory phase of A. lumbricoides larvae may result in bronchoconstriction in infected individuals. These results suggest that the Gly 16 allele confers resistance to high levels of parasitic infection in this population. An alternative explanation for the association is that it may be the result of linkage disequilibrium with other genes in the chromosome 5q31-33 region.

L40 ANSWER 30 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1999:168776 Document No.: PREV199900168776. T-cell receptor contact and MHC binding residues of a major rye grass pollen **allergen** T-cell epitope. Burton, Matthew D.; Blaher, Bella; Suphioglu, Cenk; O'Hehir, Robyn E.; Carbone, Francis R.; Rolland, Jennifer M.. Department Pathology, Immunology, Monash University Medical School, Commercial Road Prahran, 3181 VIC, Australia. Journal of Allergy and Clinical Immunology, (Feb., 1999) Vol. 103, No. 2 PART 1, pp. 255-261. print.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

AB Background: T cells are pivotal in the elicitation of allergic diseases. Analogues of T-cell epitope peptides with a modification at a T-cell receptor (TCR) contact site can alter selected T-cell effector functions. Thus the ability to modulate **allergen**-specific T-cell responses towards TH1-like by stimulation with peptide analogues may downregulate allergic inflammation. Objectives: The purpose of this study was to characterize the minimal epitope recognized by cloned T cells of a dominant Lol p 5 epitope, p105-116, and identify the critical residues involved in TCR and MHC contact. Methods: Using peptides with progressive truncation of N- and C-terminal residues in T-cell proliferation assays, we identified the core epitope recognized by cloned CD4+ T cells. An additional series of peptides with single **amino acid substitutions** were used in T-cell proliferation and live-cell MHC binding assays. Taken together, these results allowed identification of MHC binding and TCR contact residues of p105-116. Results: The core epitope of p105-116 was identified as residues 107-114. Within this core epitope, 3 residues were found to be important for MHC binding, positions 107, 110, and 112, whereas those at positions 108, 109, 110, 111, and 113 were putative TCR contact residues. Conclusions: The identification of the TCR and MHC contact residues of a dominant Lol p 5 T-cell epitope and analogues of this peptide capable of modulating T-cell responses will allow the evaluation of these peptides' potential as immunotherapeutic agents for rye grass pollen allergic disease.

L40 ANSWER 31 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1998:257635 Document No.: PREV199800257635. Antagonistic peptides specifically inhibit proliferation, cytokine production, CD40L expression, and help for IgE synthesis by Der p 1-specific human T-cell clones. Fasler, Stephan; Aversa, Gregoria; De Vries, Jan E.; Yssel, Hans [Reprint author]. INSERM U454, Hopital Arnaud de Villeneuve, 371 Ave. Doyen Gaston Giraud, 34295 Montpellier Cedex, France. Journal of Allergy and Clinical Immunology, (April, 1998) Vol. 10, No. 4 PART 1, pp. 521-530. print.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

AB Background: Allergic disorders are characterized by IgE antibody responses to a multitude of **allergens** as a result of the ability of these antibodies to specifically bind to high-affinity IgE receptors on mast cells and basophils. This interaction results in receptor activation and release of soluble mediators such as histamine and leukotrienes, which cause allergic reactions in various target organs. Because the synthesis of IgE is tightly regulated by cytokines and CD40 ligand (L) interactions, CD4+ helper T cells are obvious targets, with the aim to modulate **allergen**-induced IgE responses. Objectives: Because of the central role of **allergen**-specific T-helper type 2 (TH2) cells in the pathway leading to IgE synthesis in vitro and in vivo, we have

evaluated the possibility of inhibiting **allergen**-induced activation of these cells by using **allergen**-derived peptides that have been modified by single **amino acid substitutions**. Methods: Three cloned human TH2-like CD4+ T-cell lines, specific for Der p 1, the major **allergen** in house dust, were used in this study. Upon activation with Der p 1 or specific Der p 1-dervived wild-type peptides, these T-cell clones produce high levels of IL-4 and IL-5 and low levels of interferon-gamma and IL-2, respectively, and furthermore give help to B cells for the production of IgE in vitro. Modified synthetic peptides were generated by the introduction of single **amino acid substitutions** into two different T-cell activation-inducing epitopes on Der p 1. The effects of these modified peptides were studied in Der p 1-induced proliferation, cytokine production, and in vitro IgE production assays. Results: Several substituted Der p 1-derived peptides failed to induce T-cell proliferation, in contrast to the native peptides. In addition, some of these peptides acted as antagonists by strongly inhibiting wild-type peptide-induced proliferation as well as the production of interferon-gamma, IL-2, IL-4, and IL-5, although the production of the latter two cytokines was less affected than that of interferon-gamma, even at a 100-fold molar excess of the antagonistic peptides. In addition, the presence of an excess of each of the antagonistic peptides during the activation of Der p I-specific T-cell clones prevented induction of CD40L expression, resulting in a failure of these cells to give help to B cells for the production of IgE in vitro, even in the presence of exogenous IL-4. Conclusions: Substitution of certain amino acid residues in immunogenic Der p 1-derived peptides results in the generation of peptides that fail to induce proliferation of Der p 1-specific T-cell clones. In addition, these modified peptides have strong antagonistic activities on Der p 1-induced proliferation, cytokine production, and CD40L expression by **allergen**-specific T-cell clones as well as on T cell-mediated IgE production by B cells. These findings suggest that modified peptides interfere with **allergen**-induced activation of T cells, including the production of cytokines and the expression of surface molecules important for successful T cell-B cell interactions, and may therefore have therapeutic potential by inhibiting the expansion and function of **allergen**-specific TH2 cells.

L40 ANSWER 32 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1999:129223 Document No.: PREV199900129223. Molecular structure of the major birch **allergen** and the effect of surface exposed **amino acid substitution** on IgE binding. Larsen, J. N. [Reprint author]; Gajhede, M.; Ipsen, H. [Reprint author]; Spangfort, M. D. [Reprint author]; Van Neerven, R. J. J. [Reprint author]; Schou, C. [Reprint author]; Lowenstein, H. [Reprint author]. Alk-Abello, Horsholm, Denmark. European Respiratory Journal, (Sept., 1998) Vol. 12, No. SUPPL. 28, pp. 275S. print.
Meeting Info.: European Respiratory Society Annual Congress. Geneva, Switzerland. September 19-23, 1998. The European Respiratory Society.
CODEN: ERJOEI. ISSN: 0903-1936. Language: English.

L40 ANSWER 33 OF 45 MEDLINE on STN DUPLICATE 10
1998413866. PubMed ID: 9742934. Engineering of hypoallergenic mutants of the Brassica pollen **allergen**, Bra r 1, for immunotherapy. Okada T; Swoboda I; Bhalla P L; Toriyama K; Singh M B. (Laboratory of Plant Breeding and Genetics, Graduate School of Agricultural Science, Tohoku University, Sendai, Japan. ) FEBS letters, (1998 Sep 4) 434 (3) 255-60. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.
AB The Brassica pollen **allergen** Bra r 1 belongs to a new family of Ca2+-binding proteins, characterized by the presence of two potential EF-hand calcium-binding domains. Disruption of these EF-hand motifs by **amino acid substitutions** demonstrated that both domains of Bra r 1 constitute functional Ca2+-binding sites. Calcium-binding deficient mutants displayed significantly reduced

IgE-binding activity. Injection of these mutated Bra r 1 variants into a murine model system showed that mouse IgG raised against the mutants recognized native Bra r 1 in Brassica pollen extracts suggesting the potential use of the engineered **allergens** for effective immunotherapy.

L40 ANSWER 34 OF 45 MEDLINE on STN
1998022765. PubMed ID: 9354640. A single **amino acid substitution** in ribonucleolytic toxin restrictocin abolishes its specific substrate recognition activity. Nayak S K; Batra J K. (Immunochemistry Laboratory, National Institute of Immunology, New Delhi, India. ) Biochemistry, (1997 Nov 4) 36 (44) 13693-9. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.
AB Restrictocin is a small basic protein produced by the fungus Aspergillus restrictus. It potently inhibits protein synthesis in eukaryotic cells by specifically cleaving a single phosphodiester bond in 28S rRNA. A histidine residue at position 49 in restrictocin has been implicated in its active site. A mutant of restrictocin in which the histidine at position 49 was changed to an alanine was constructed by site-directed mutagenesis, and the protein was expressed in Escherichia coli. The mutant and the wild type proteins were found to be structurally identical. Unlike restrictocin, the restrictocin H49A mutant did not cleave the ribosomal RNA specifically at the target phosphodiester bond; instead, it extensively degraded the RNA substrate with altered specificity. The mutant exhibited a high ribonuclease activity compared to restrictocin on yeast tRNA, and poly(U) and poly(C). The mutant also poorly inhibited protein synthesis in eukaryotic cells as well as in a cell free system. We therefore propose that histidine 49 of restrictocin is not involved per se in the enzymatic activity; however, it does play a crucial role in the specific recognition of the target sequence by restrictocin.

L40 ANSWER 35 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1997:360802 Document No.: PREV199799652735. Comparison of natural and recombinant isoforms of grass pollen **allergens.** Petersen, Arnd [Reprint author]; Grobe, Kay; Lindner, Buko; Schlaak, Max; Becker, Wolf-Meinhard. Forschungszentrum Borstel, Div. Allergol., Parkallee 22, D-23845 Borstel, Germany. Electrophoresis, (1997) Vol. 18, No. 5, pp. 819-825.
CODEN: ELCTDN. ISSN: 0173-0835. Language: English.
AB More than 95% of grass pollen allergic patients possess IgE antibodies against grass group I, a heterogeneous group of glycoproteins found in all temperate grasses. We studied the structural variability of the group I **allergens** in single species and among different grasses. By 2-DE blotting using patients' IgE and monoclonal antibodies, we detected IgE-reactive isoforms with molecular masses between 32 and 37 kDa and focusing in a wide pI ranging from 4.7 to 7.6. While the group I **allergens** of timothy grass (Phl p 1) were composed of 37 and 35 kDa components, only single isoforms were found for ryegrass (Lol p 1) and velvet grass (Hol l 1): 32 and 34 kDa, respectively. By N-terminal microsequencing we determined single **amino acid substitutions** in different-sized group I **allergens.** The post-translational modifications (one N-glycosylation site, two hydroxylated proline residues and seven cysteine residues for potential disulfide formations), which contribute to IgE reactivity, were identical in all. From the cDNA sequences we deduced protein sequence homologies gt 90%, a result which might explain the high IgE cross-reactivity among the grasses. In order to test whether recombinant group I grass **allergens** can act as substitutes for the natural forms, we expressed rPhl p 1 in E. coli and in P. pasteuris. 2-DE immunoblotting again demonstrated a microheterogeneity in molecular mass and pI. While the E. coli products were free from post-translational modifications, rPhl p 1 from Pichia is a heterogeneous glycoprotein fraction with a carbohydrate content of about 15%. This rPhl p 1 is hyperglycosylated compared to the nPhl p 1, which only has a 5% carbohydrate content.

L40 ANSWER 36 OF 45 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 11

97171536 EMBASE Document No.: 1997171536. Localization of antigenic sites on Der p 2 using oligonucleotide- directed mutagenesis targeted to predicted surface residues. Smith A.M.; Chapman M.D.. Dr. A.M. Smith, Asthma and Allergic Diseases Center, Univ. of Virginia School of Medicine, Box 225, Charlottesville, VA 22908, United States. Clinical and Experimental Allergy 27/5 (593-599) 1997.
Refs: 27.
ISSN: 0954-7894. CODEN: CLEAEN. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB Background: Understanding the molecular nature of **allergen** -antibody interactions is important to understanding the mechanism of conventional immunotherapy as well as to designing alternative immunotherapeutic strategies. Many important **allergens** have been cloned and expressed, making it possible to apply recombinant DNA techniques to dissect antigenic determinants. Objective: The aim of this study was to use predictive algorithms and site-directed mutagenesis to investigate monoclonal antibody and IgE antibody epitopes of the major house dust mite **allergen** Der p 2. Methods: Computer algorithms were used to assess the primary amino acid sequence of Der p 2 and to identify regions of hydrophilic and flexible sequence. Subsequently, site-directed mutagenesis was used to generate **amino acid substitutions** at hydrophilic residues at positions 44-46 and at position 100. The variants were tested in a competitive inhibition ELISA with four group 2-specific murine monoclonal antibodies and with human IgE antibody from mite allergic patients. Results: Conservative **amino acid substitutions** at position 44-46 did not distinguish IgE antibody epitopes, but did suggest that these residues are involved in the epitope defined by one monoclonal antibody, 15E11. Non-conservative substitution of proline at this position reduced binding to all four monoclonal antibodies, as well as IgE antibody, by 50-80%. Point mutants at position 100 mapped the epitopes of two monoclonal antibodies, 7A1 and 13A4, previously shown to bind the same region of Der p 2. In addition, the two variants tested at this position showed distinct inhibition curves with these two monoclonal antibodies indicating differences in fine specificity. Conclusions: Using predictive algorithms, in the absence of tertiary structural information, we have been able to localize important B cell determinants on Der p 2. The results suggest that it is possible to modulate antibody recognition of **allergens** using site-directed mutagenesis and that this approach may provide a new strategy for **allergen** specific immunotherapy.

L40 ANSWER 37 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

1997:144056 Document No.: PREV199799443259. 3-D Structure and epitopes of birch pollen **allergen** Bet v 1. Spangfort, M. D. [Reprint author]; Gajhede, M.; Ipsen, H.; Larsen, J. N.; Van Neerven, R. J. J.; Schou, C.; Osmark, P.; Poulsen, F. M.; Lowenstein, H.. Horsholm, Denmark. Journal of Allergy and Clinical Immunology, (1997) Vol. 99, No. 1 PART 2, pp. S133.
Meeting Info.: Joint Meeting of the American Academy of Allergy, Asthma and Immunology, the American Association of Immunologists and the Clinical Immunology Society. San Francisco, California, USA. February 21-26, 1997.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L40 ANSWER 38 OF 45 MEDLINE on STN

96435212. PubMed ID: 8838098. Molecular biological analysis of house dust mite **allergens**. Okudaira H; Okumura Y; Sato G. (Department of Medicine and Physical Therapy, University of Tokyo. ) Nippon rinsho. Japanese journal of clinical medicine, (1996 Feb) 54 (2) 466-71. Ref: 10.
Journal code: 0420546. ISSN: 0047-1852. Pub. country: Japan. Language: Japanese.

AB Using a host-vector system of Escherichia coli, we could produce one of major house dust mite **allergens**, Der f II in large quantity for therapeutic and diagnostic purposes. About 5 mg of purified and

biologically active rDer f II was obtained from one liter culture, which was corresponding to the amount in about 30 g of live mite. The rDer f II was almost identical with native Der f II with respects to biological and physicochemical view points. Native mite Der f II is a mixture of several kinds of Der f II molecule with a few **amino acid substitutions**, which were due to polymorphisms among individual mite gene sequence. We had cloned three kinds of Der f II cDNAs from mite culture and expressed in E. coli and prepared three kinds of rDer f II in this system. As a result of comparison of IgE binding activity among three rDer f IIs and nDer f II, there was no significant difference observed.

L40 ANSWER 39 OF 45 MEDLINE on STN DUPLICATE 12
96319509. PubMed ID: 8768806. IgE responsiveness to Dermatophagoides farinae in young asthmatic children: IgE binding study using recombinant **allergens** of Der f1, Der f2 and mutant proteins of Der f2. Noguchi E; Shibasaki M; Nishiyama C; Okumura Y; Takita H. (Department of Pediatrics, Institute of Clinical Medicine, University of Tsukuba, Japan. ) International archives of allergy and immunology, (1996 Aug) 110 (4) 380-7. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB IgE reactivities to two recombinant (r) **allergens**, rDer f1 and rDer f2, synthetized by cDNA clones from Dermatophagoides farinae (Df) were analyzed using sera of Df-sensitive asthmatic patients of varying ages. Positive RAST responses to rDer f1 and rDer f2 were found in 88 and 80%, respectively, with sera from young asthmatic children aged 0-1, positive RAST rates to these antigens increased to 100% up to 5 years of age. There was a significant correlation between RAST levels of rDer f1 and rDer f2 in these young children aged 0-1. IgE reactivities to four mutant proteins of rDer f2, which have at least one **amino acid substitution**, were similarly examined in the asthmatic sera. IgE reactivity to D7A, in which Asp7 was replaced by Ala, was reduced to 30-60% compared to the rDer f2. IgE binding to C8/119S, in which both Cys8 and Cys119 were replaced by Ser, lacking a disulfide bond between Cys8 and Cys119, was reduced almost to a background level at all ages. In contrast, A72L and A120L, in which Ala72 and Ala120 were substituted for Leu, respectively, almost retained the same IgE binding activity as the rDer f2 at all ages. These results suggest that Der f1 and Der f2 are important antigens associated with early sensitization to house dust mite in young asthmatic children. In addition, the disulfide bond between Cys8 and Cys119 and the N-terminal region including the 7th amino acid residue are considered to maintain IgE epitopes of the Der f2 **allergen.**

L40 ANSWER 40 OF 45 MEDLINE on STN DUPLICATE 13
96162074. PubMed ID: 8563488. Comparative analysis of the genes encoding group 3 **allergens** from Dermatophagoides pteronyssinus and Dermatophagoides farinae. Smith W A; Thomas W R. (TVW Telethon Research Institute for Child Health, Perth, Australia. ) International archives of allergy and immunology, (1996 Feb) 109 (2) 133-40. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB Group 3 **allergens** of the genus Dermatophagoides represent one of the major groups of house dust mite **allergens.** The cDNA sequence data for Der p 3, in combination with both N-terminal amino acid sequences and substrate affinity data, have confirmed that the group 3 **allergens** are trypsin-like proteases. Using the information from the Der p 3 P3WS1 cDNA clone, genes encoding both Der f3 and Der p 3 have now been amplified by the polymerase chain reaction and analysed. Two Der f3 clones and three Der p 3 genomic clones were sequenced. Each of the clones contained a single small intron and encoded a mature protein of 233 amino acids. The nucleotide sequence was identical for both Der f3 clones. There was 81% identity between the Der f3 sequence and the original Der p 3 P3WS1 clone. The calculated molecular weight of Der f3 was 25.27 kDa compared to 24.98 kDa for Der p 3. All the amino acid residues required for the catalytic activity and the substrate specificity

were conserved between the two homologues. The coding sequences of two of the three Der p 3 genomic clones were identical to the original Der p 3 P3WS1 clone with the third having nucleotide changes resulting in four non-conservative **amino acid substitutions** in the mature protein. These substitutions resulted in a molecule with a slightly larger molecular weight and a more acidic pI value than the original Der p 3 clone. This third Der p 3 genomic clone is, therefore, an isoform of the Der p 3 P3WS1 clone and is classified as an isovariant of the **allergen.** The nucleotide sequence data presented are the first reported for Der f 3. The Der f 3 gene, like the Der p 3 gene, encoded a trypsin-like protease, but with a slightly larger molecular weight.

L40 ANSWER 41 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1996:125261 Document No.: PREV199698697396. An allergenic polypeptide representing a variable region of hsp 70 cloned from a cDNA library of Cladosporium herbarum. Zhang, L.; Muradia, G.; De Vouge, M. W.; Rode, H.; Vijay, H. M. [Reprint author]. Life Sci. Div., Bureau Drug Res., Health Canada, Postal Locator 2201C, Ottawa, ON K1A 0L2, Canada. Clinical and Experimental Allergy, (1996) Vol. 26, No. 1, pp. 88-95.
ISSN: 0954-7894. Language: English.

AB Background: Extracts of Cladosporium herbarum, a major source of fungal aeroallergens, exhibit a complex profile of IgE-binding proteins. Yields of conventionally purified **allergens** from this mold have been insufficient to permit further molecular analyses. Objective: To enhance and simplify the purification of **allergens** from C. herbarum, we have sought to use recombinant DNA techniques to clone, identify and bacterially express immunoselected C. herbarum **allergens.** Methods: We constructed a cDNA library in lambda-ZAP II using mRNA isolated from C. herbarum. From this library, phage clones encoding a new **allergen** were immunoselected using pooled human atopic IgE. The cloned cDNA was excised from the phage vector as a recombinant pBluescript II SK-phagemid and sequenced. Expression of the recombinant **allergen** was carried out in E. coli XL1-blue transformants of the phagemid. Bacterial lysates from cells induced to express the cloned **allergen** were immunoblotted and probed with individual human atopic IgEs. Results: The cDNA clone encodes a 278 amino acid polypeptide homologous to the C-terminal portion of 70 kDa heat shock protein (hsp 70). The polypeptide possesses features common to other hsps 70, i.e. a similar hydropathic profile and a variable C-terminal region with conserved sequence at the very C-terminus. Binding of the recombinant peptide to IgE from 38% of atopic sera or plasma from individuals allergic to C. herbarum was demonstrated. Conclusion: These results indicate that **amino acid substitutions** are relatively conserved even in the variable C-terminal regions of hsp 70 species. Thus, this study should draw attention to the possibility of induction of anaphylactic responses in a sensitized individual when hsp 70 from any pathogenic species is administered for vaccination.

L40 ANSWER 42 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1996:101402 Document No.: PREV199698673537. Single **amino acid substitutions** on a Japanese cedar pollen **allergen** (Cry j 1)-derived peptide induced alterations in human T cell responses and T cell receptor antagonism. Ikagawa, Shuji; Matsushita, Sho; Chen, Yu-Zhen; Ishikawa, Takeru; Nishimura, Yasuharu [Reprint author]. Div. Immunogenet., Dep. Neurosci. Immunol., Kumamoto Univ. Graduate Sch. Med. Sci., Honjo 2-2-1, Kumamoto 860, Japan. Journal of Allergy and Clinical Immunology, (1996) Vol. 97, No. 1 PART 1, pp. 53-64.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

AB We generated T cell clones specific to a Japanese cedar pollen **allergen** (Cry j 1) and investigated effects of altered T cell receptor (TCR) ligand on changes of T cell responses. One of these Cry j 1-specific T cell clones established from patients with Japanese cedar pollinosis, ST1.9, recognized an antigenic peptide Cry j 1 p335-346 in the context of HLA-DRA+DRB3*0301 molecules and secreted interleukin-4

dominantly, with a smaller amount of interferon-gamma. ST1.9 represented one of the major T cell clones specific to Cry j 1 in the donor, because a short-term cultured polyclonal T cell line specific to Cry j 1 exhibited the same character as the ST1. 9. We synthesized various analog peptides derived from Cry j 1 p335-346 with single **amino acid substitutions** and determined key residues for interactions between TCR of ST1. 9 and HLA-DR molecules. We also analyzed changes in the responses of ST1.9 to Cry j 1 p335-346-derived analog peptides. Of interest was that the substitution of 339threonine to valine resulted in a significant increase in interferon-gamma production, with no remarkable changes either in proliferative response or interleukin-4 production. Analog peptides carrying the substitutions of 339threonine to glycine or glutamine revealed TCR antagonism, without changes in their binding affinities to the DR molecule. Therefore single **amino acid substitutions** on an **allergen** peptide carrying the T cell epitope may suppress helper-T-dependent class switch pressure to IgE in B cells either by inducing increased interferon-gamma production or by inhibiting proliferative responses in helper-T cells.

L40 ANSWER 43 OF 45 MEDLINE on STN

95337757. PubMed ID: 7542079. The significance of isoallergenic variations in present and future specific immunotherapy. Lowenstein H; Sparholt S H; Klysner S S; Ipsen H; Larsen J N. (ALK Laboratories, Horsholm, Denmark. ) International archives of allergy and immunology, (1995 May-Jun) 107 (1-3) 285-9. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB The isoallergenic variation of the tree pollen major **allergens** has been studied by 2D gel electrophoresis, and by analysis of several recombinant clones. The studies have included both antibody-based and T cell stimulation assays. Bet v 1, the major **allergen** of birch, forms at least 24 spots when conventional extracts are analyzed by 2D gel electrophoresis. Comparison of Bet v 1-encoding DNA sequences reveals a considerable number of **amino acid substitutions.** This sequence variation can theoretically account for the number of spots observed in 2D gels. Whereas pools of serum from allergic individuals and monospecific antibodies raised in rabbits bind to most, if not all spots in 2D gels, analyses of individual serum and/or murine monoclonal antibodies show individual patterns of reactivity with various subsets of spots. These observations point to a model in which **amino acid substitutions** induce local perturbations of the **allergen** surface, causing differences in epitope structure. Furthermore, analysis of pollen from individual trees shows that each tree produces individual subsets of Bet v 1 spots. When analyzed in stimulation assays, T cell clones also display differences in reactivity to different isoallergens. In conclusion, we have shown that Bet v 1 is heterogeneous, and that individual trees produce various subsets of isoallergens which display differences in reactivity both towards antibodies and T cells. A careful selection of isoform may therefore be of major importance if recombinant **allergens** or synthetic peptides are to be used for conventional immunotherapy.

L40 ANSWER 44 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN

1994:678862 Document No. 121:278862 Allergenic proteins and peptides from dog dander and nucleic acids encoding them and their uses. Morgenstern, Jay P.; Konieczny, Andrzej; Bizinkauskas, Christine B.; Brauer, Andrew W. (Immulogic Pharmaceutical Corp., USA). PCT Int. Appl. WO 9416068 A2 19940721, 129 pp. DESIGNATED STATES: W: AU, CA, JP, KR, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1993-US12468 19931230. PRIORITY: US 1992-999712 19921231; US 1993-156549 19931122.

AB CDNAs encoding the Canis familiaris **allergens** Can f I or Can f II are cloned and expressed. The nucleic acids can be used as probes to detect the presence of Can f I or Can f II nucleic acid in a sample or for the manufacture of the peptides for use in the diagnosis or treatment of sensitivity to dander. A cDNA for the Can f I **allergen** was

cloned from parotid gland mRNA by preparing a partial cDNA using primers derived from N-terminal sequencing and obtaining a full-length cDNA by RACE. A cDNA for the Can f II **allergen** was cloned by screening a parotid gland cDNA bank in λgt10 with PCR products prepared using primers derived from an extended N-terminal sequence. Manufacture of the **allergens** in Escherichia coli and in NIH 3T3 cells using the expression vector pJ7Ω, using a (His)6 label for affinity purification in both cases, is demonstrated. Antibody binding to the proteins manufactured in **animal** cells on Western blots was weak. The allergenic activity appears to be located at many sites throughout the proteins.

L40 ANSWER 45 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN
1993:579195 Document No. 119:179195 T cell epitopes of the major **allergens** from Dermatophagoides (house dust mite). Garman, Richard D.; Greenstein, Julia L.; Kuo, Mei Chang; Rogers, Bruce L. (Immulogic Pharmaceutical Corp., USA). PCT Int. Appl. WO 9308279 A1 19930429, 176 pp. DESIGNATED STATES: W: AU, CA, FI, HU, JP, KR, NO; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1992-US8637 19921015. PRIORITY: US 1991-777859 19911016; US 1992-881396 19920508.
AB The 4 **allergens** were immunoaffinity-purified from spent mite culture media. Recombinant **allergens** were also prepared by cloning and expressing the cDNA in BL21 cells; amino acid sequence polymorphisms were discovered. T cell epitopic studies and cross reactivity studies are shown. There was no detectable IgE reactivity to any of 56 T cell epitopic peptides screened.

=> s l12 and latexes
L41 0 L12 AND LATEXES

=> s l12 and natural latexes
L42 0 L12 AND NATURAL LATEXES

=> s (burks w?/au or helm r?/au or cockrell g?/au or bannon g?/au or stanley s?/au or shin d?/au or sampson h?/au or compadre c?/au or huangs?/au or maleki s?/au or kopper r?/au)
L43 12279 (BURKS W?/AU OR HELM R?/AU OR COCKRELL G?/AU OR BANNON G?/AU OR STANLEY S?/AU OR SHIN D?/AU OR SAMPSON H?/AU OR COMPADRE C?/AU OR HUANGS?/AU OR MALEKI S?/AU OR KOPPER R?/AU)

=> s l43 and modified allergen
L44 11 L43 AND MODIFIED ALLERGEN

=> dup remove l44
PROCESSING COMPLETED FOR L44
L45 6 DUP REMOVE L44 (5 DUPLICATES REMOVED)

=> d l45 1-6 cbib abs

L45 ANSWER 1 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN
2003:855391 Document No. 139:363577 Modified anaphylactic food allergens with reduced IgE-binding ability for decreasing clinical reaction to allergy. Caplan, Michael J.; Sosin, Howard B.; **Sampson, Hugh**; **Bannon, Gary A.**; Burks, A. Wesley; **Cockrell, Gael**; **Compadre, Cesar M.**; Connaughton, Cathie; **Helm, Ricki M.**; King, Nina E.; **Kopper, Randall A.**; **Maleki, Soheila J.**; Rabjohn, Patrick A.; **Shin, David S.**; Stanley, J. Steven (USA). U.S. Pat. Appl. Publ. US 2003202980 A1 20031030, 194 pp., Cont.-in-part of U.S. Ser. No. 494,096. (English). CODEN: USXXCO. APPLICATION: US 2002-100303 20020318. PRIORITY: US 95-PV9455; 19951229; US 96-717933; 19960923; US 98-PV73283; 19980131; US 98-PV74633; 19980213; US 98-PV74624; 19980213; US 98-PV74590; 19980213; US 98-106872; 19980629; US 98-141220; 19980827; US 98-191593; 19981113; US 99-241101; 19990129; US 99-240557; 19990129; US 99-248674; 19990211; US 99-248673; 19990211; US 99-PV122560;

19990302; US 99-PV122565; 19990302; US 99-PV122566; 19990302; US 99-PV122450; 19990302; US 99-PV122452; 19990302; US 99-267719; 19990311; US 2000-494096; 20000128.

AB It has been determined that allergens, which are characterized by both humoral (IgE) and cellular (T-cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by altering as little as a single amino acid within the protein, preferably a hydrophobic residue towards the center of the IgE epitope, to eliminate IgE binding. Addnl. or alternatively a **modified allergen** with reduced IgE binding may be prepared by disrupting one or more of the disulfide bonds that are present in the natural allergen. The disulfide bonds may be disrupted chemical, e.g., by reduction and alkylation or by mutating one or more cysteine residues present in the primary amino acid sequence of the natural allergen. In certain embodiments, **modified allergens** are prepared by both altering one or more linear IgE epitopes and disrupting one or more disulfide bonds of the natural allergen. In certain embodiments, the methods of the present invention allow allergens to be modified while retaining the ability of the protein to activate T-cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The immunotherapeutics can be prepared in transgenic plants or animals; and administered in injection, aerosol, sublingual or topical form. The immunotherapeutics can also be encoded in gene for gene therapy and delivered by injecting into muscle or skin to induce tolerance. The Examples provided herein use peanut allergens to illustrate applications of the invention.

L45 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN

2003:906632 Correction of: 2002:736063 Document No. 139:349665 Correction of: 137:277814 Modified anaphylactic food allergens with reduced IgE-binding ability for decreasing clinical reaction to allergy. Caplan, Michael; Sosin, Howard; **Sampson, Hugh**; **Bannon, Gary A.**; **Burks, Wesley A.**; **Cockrell, Gael**; **Compadre, Cesar M.**; Connaughton, Cathie; **Helm, Ricki M.**; King, Nina E.; **Kopper, Randall A.**; **Maleki, Sohelia J.**; Rabjohn, Patrick A.; **Shin, David S.**; Stanley, J. Steven (Panacea Pharmaceuticals, USA; et al.). PCT Int. Appl. WO 2002074250 A2 20020926, 299 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US9108 20020318. PRIORITY: US 2001-PV276822 20010316.

AB It has been determined that allergens, which are characterized by both humoral (IgE) and cellular (T-cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by altering as little as a single amino acid within the protein, preferably a hydrophobic residue towards the center of the IgE epitope, to eliminate IgE binding. Addnl. or alternatively a **modified allergen** with reduced IgE binding may be prepared by disrupting one or more of the disulfide bonds that are present in the natural allergen. The disulfide bonds may be disrupted chemical, e.g., by reduction and alkylation or by mutating one or more cysteine residues present in the primary amino acid sequence of the natural allergen. In certain embodiments, **modified allergens** are prepared by both altering one or more linear IgE epitopes and disrupting one or more disulfide bonds of the natural allergen. In certain embodiments, the methods of the present invention allow allergens to be modified while retaining the ability of the protein to activate T-cells, and, in some embodiments by not significantly

altering or decreasing IgG binding capacity. The Examples provided herein use peanut allergens to illustrate applications of the invention.

L45 ANSWER 3 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 1
2002:301398 Document No.: PREV200200301398. Immunotherapy for peanut allergy using **modified allergens** and a bacterial adjuvant.
Stanley, Joseph Steve [Reprint author]; Buzen, Fred [Reprint author]; **Cockrell, Gael** [Reprint author]; West, Mike [Reprint author]; Srivastava, Kamal D.; Li, X. M.; **Sampson, Hugh A.**; **Burks, Wesley** [Reprint author]; **Bannon, Gary A.** [Reprint author].
University of Arkansas, Little Rock, AR, USA. Journal of Allergy and Clinical Immunology, (January, 2002) Vol. 109, No. 1 Supplement, pp. S93. print.
Meeting Info.: 58th Annual Meeting of the American Academy of Allergy, Asthma and Immunology. New York, NY, USA. March 01-06, 2002. American Academy of Allergy, Asthma, and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L45 ANSWER 4 OF 6 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2002:530650 The Genuine Article (R) Number: 563MD. Modification of peanut allergen Ara h 3: Effects on IgE binding and T cell stimulation. Rabjohn P; West C M; Connaughton C; **Sampson H A**; **Helm R M (Reprint)**; Burks A W; **Bannon G A.** Univ Arkansas Med Sci, ACHRI, Dept Biochem & Mol Biol, Slot 512, 1120 Marshall St, Little Rock, AR 72202 USA (Reprint); Univ Arkansas Med Sci, ACHRI, Dept Biochem & Mol Biol, Little Rock, AR 72202 USA; Univ Arkansas Med Sci, ACHRI, Dept Pediat, Little Rock, AR 72202 USA; Mt Sinai Sch Med, Dept Pediat, New York, NY USA. INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY (MAY 2002) Vol. 128, No. 1, pp. 15-23. Publisher: KARGER. ALLSCHWILERSTRASSE 10, CH-4009 BASEL, SWITZERLAND. ISSN: 1018-2438. Pub. country: USA. Language: English.
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
AB Background: Peanut allergy is a major health concern due to the increased prevalence, potential severity, and chronicity of the reaction. The cDNA encoding a third peanut allergen, Ara h 3, has been previously cloned and characterized. Mutational analysis of the Ara h 3 IgE-binding epitopes with synthetic peptides revealed that single amino acid changes at critical residues could diminish IgE binding. Methods: Specific oligonucleotides were used in polymerase chain reactions to modify the cDNA encoding Ara h 3 at critical IgE binding sites. Four point mutations were introduced into the Ara h 3 cDNA at codons encoding critical amino acids in epitopes 1, 2, 3 and 4. Recombinant modified proteins were used in SDS-PAGE/Western IgE immunoblot, SDS-PAGE/Western IgE immunoblot inhibition and T cell proliferation assays to determine the effects of these changes on in vitro clinical indicators of peanut hypersensitivity. Results: Higher amounts of modified Ara h 3 were required to compete with the wild-type allergen for peanut-specific serum IgE. Immunoblot analysis with individual serum IgE from Ara-h-3-allergic patients showed that IgE binding to the modified protein decreased similar to35-85% in comparison to IgE binding to wildtype Ara h 3. Also, the modified Ara h 3 retained the ability to stimulate T cell activation in PBMCs donated by Ara-h-3-allergic patients. Conclusions: The engineered hypoallergenic Ara h 3 variant displays two characteristics essential for recombinant allergen immunotherapy; it has a reduced binding capacity for serum IgE from peanut-hypersensitive patients and it can stimulate T-cell proliferation and activation. 

L45 ANSWER 5 OF 6 MEDLINE on STN DUPLICATE 2
2001262411. PubMed ID: 11306930. Engineering, characterization and in vitro efficacy of the major peanut allergens for use in immunotherapy.
**Bannon G A**; **Cockrell G**; Connaughton C; West C M; **Helm R**; Stanley J S; King N; Rabjohn P; **Sampson H A**; Burks A W. (Department of Biochemistry and Molecular Biology, Arkansas Children's Hospital Research Institute, Little Rock 72205, USA..

bannongarya@exchnage.uams.edu) . International archives of allergy and immunology, (2001 Jan-Mar) 124 (1-3) 70-2. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Numerous strategies have been proposed for the treatment of peanut allergies, but despite the steady advancement in our understanding of atopic immune responses and the increasing number of deaths each year from peanut anaphylaxis, there is still no safe, effective, specific therapy for the peanut-sensitive individual. Immunotherapy would be safer and more effective if the allergens could be altered to reduce their ability to initiate an allergic reaction without altering their ability to desensitize the allergic patient. METHODS: The cDNA clones for three major peanut allergens, Ara h 1, Ara h 2, and Ara h 3, have been cloned and characterized. The IgE-binding epitopes of each of these allergens have been determined and amino acids critical to each epitope identified. Site-directed mutagenesis of the allergen cDNA clones, followed by recombinant production of the **modified allergen**, provided the reagents necessary to test our hypothesis that hypoallergenic proteins are effective immunotherapeutic reagents for treating peanut-sensitive patients. Modified peanut allergens were subjected to immunoblot analysis using peanut-positive patient sera IgE, T cell proliferation assays, and tested in a murine model of peanut anaphylaxis. RESULTS: In general, the **modified allergens** were poor competitors for binding of peanut-specific IgE when compared to their wild-type counterpart. The **modified allergens** demonstrated a greatly reduced IgE-binding capacity when individual patient serum IgE was compared to the binding capacity of the wild-type allergens. In addition, while there was considerable variability between patients, the **modified allergens** retained the ability to stimulate T cell proliferation. CONCLUSIONS: These **modified allergen** genes and proteins should provide a safe immunotherapeutic agent for the treatment of peanut allergy. Copyright 2001 S. Karger AG, Basel

L45 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN

1999:495393 Document No. 131:143513 Methods and reagents for decreasing allergic reactions. Sosin, Howard; **Bannon, Gary A.**; Burks, A. Wesley, Jr.; **Sampson, Hugh A.** (University of Arkansas, USA; Mt. Sinai School of Medicine, University of New York). PCT Int. Appl. WO 9938978 A1 19990805, 46 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US2031 19990129. PRIORITY: US 1998-PV73283 19980131; US 1998-PV74590 19980213; US 1998-PV74624 19980213; US 1998-PV74633 19980213; US 1998-141220 19980827.

AB It has been determined that allergens, which are characterized by both humoral (IgE) and cellular (T cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by masking the site with a compound that prevents IgE binding or by altering as little as a single amino acid within the protein, most typically a hydrophobic residue towards the center of the IgE-binding epitope, to eliminate IgE binding. The method allows the protein to be altered as minimally as possible, other than within the IgE-binding sites, while retaining the ability of the protein to activate T cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The examples use peanut allergens to demonstrate alteration of IgE binding sites. The critical amino acids within each of the IgE binding epitopes of the peanut protein that are important to Ig binding have been determined Substitution of even a single amino acid within each of the epitopes led to loss of IgE binding. Although the epitopes shared no common amino acid sequence motif, the hydrophobic residues located in the center of the epitope appeared to be

most critical to IgE binding.

=> s l43 and allergen
L46 823 L43 AND ALLERGEN

=> dup remove l46
PROCESSING COMPLETED FOR L46
L47 403 DUP REMOVE L46 (420 DUPLICATES REMOVED)

=> s l47 and IgE epitope
L48 6 L47 AND IGE EPITOPE

=> dup remove l48
PROCESSING COMPLETED FOR L48
L49 6 DUP REMOVE L48 (0 DUPLICATES REMOVED)

=> d l49 1-6 cbib abs

L49 ANSWER 1 OF 6 MEDLINE on STN
2004202343. PubMed ID: 15100687. Microarray immunoassay: association of clinical history, in vitro IgE function, and heterogeneity of allergenic peanut epitopes. Shreffler Wayne G; Beyer Kirsten; Chu Te-Hua Tearina; Burks A Wesley; **Sampson Hugh A.** (Jaffe Food Allergy Institute, Division of Allergy and Immunology, Department of Pediatrics, Mount Sinai Medical Center, Box 1198, One Gustave L. Levy Place, New York, NY 10029, USA. ) Journal of allergy and clinical immunology, (2004 Apr) 113 (4) 776-82. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: **IgE epitope** mapping of food **allergens** is a prerequisite for engineering hypoallergenic immunotherapeutic agents and might reveal basic information regarding a patient's immune response. Mapping of large numbers of epitopes by using individual patient sera has been impractical with current techniques. OBJECTIVE: We sought to develop a peptide microarray-based immunoassay to map peanut epitopes by using microliter quantities of serum. METHODS: A set of 213 overlapping 20-residue peptides was synthesized corresponding to the primary sequences of Ara h 1, Ara h 2, and Ara h 3. These were arrayed in triplicate along with the corresponding recombinant proteins onto glass slides and used for immunolabeling. RESULTS: Seventy-seven patient and 15 control sera were analyzed. The majority of patients (97%) had specific IgE to at least one of the recombinant **allergens**, and 87% had detectable IgE to sequential epitopes. Microarray mapping correlated well with previous studies. However, the analysis of individual patients revealed remarkable heterogeneity in the number and patterns of epitope recognition. High epitope diversity was found in patients with a history of more severe allergic reactions. Also, sensitization of effector cells with more diverse IgE antibodies conferred greater reactivity to specific **allergen.** CONCLUSIONS: The protein microarray immunoassay confirmed that Ara h 1, Ara h 2, and Ara h 3 are major peanut **allergens** and allows for parallel epitope analysis. This has led to the discovery of an additional important epitope of Ara h 1 and the recognition of a high degree of patient heterogeneity. This qualitative difference in epitope diversity might provide prognostic information about the patient.

L49 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN
2003:855391 Document No. 139:363577 Modified anaphylactic food **allergens** with reduced IgE-binding ability for decreasing clinical reaction to allergy. Caplan, Michael J.; Sosin, Howard B.; **Sampson, Hugh**; **Bannon, Gary A.**; Burks, A. Wesley; **Cockrell, Gael**; **Compadre, Cesar M.**; Connaughton, Cathie; **Helm, Ricki M.**; King, Nina E.; **Kopper, Randall A.**; **Maleki, Soheila J.**; Rabjohn, Patrick A.; **Shin, David S.**; Stanley, J. Steven (USA). U.S. Pat. Appl. Publ. US 2003202980 A1 20031030, 194

pp., Cont.-in-part of U.S. Ser. No. 494,096. (English). CODEN: USXXCO. APPLICATION: US 2002-100303 20020318. PRIORITY: US 95-PV9455; 19951229; US 96-717933; 19960923; US 98-PV73283; 19980131; US 98-PV74633; 19980213; US 98-PV74624; 19980213; US 98-PV74590; 19980213; US 98-106872; 19980629; US 98-141220; 19980827; US 98-191593; 19981113; US 99-241101; 19990129; US 99-240557; 19990129; US 99-248674; 19990211; US 99-248673; 19990211; US 99-PV122560; 19990302; US 99-PV122565; 19990302; US 99-PV122566; 19990302; US 99-PV122450; 19990302; US 99-PV122452; 19990302; US 99-267719; 19990311; US 2000-494096; 20000128.

AB It has been determined that **allergens**, which are characterized by both humoral (IgE) and cellular (T-cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by altering as little as a single amino acid within the protein, preferably a hydrophobic residue towards the center of the **IgE epitope**, to eliminate IgE binding. Addnl. or alternatively a modified **allergen** with reduced IgE binding may be prepared by disrupting one or more of the disulfide bonds that are present in the natural **allergen.** The disulfide bonds may be disrupted chemical, e.g., by reduction and alkylation or by mutating one or more cysteine residues present in the primary amino acid sequence of the natural **allergen.** In certain embodiments, modified **allergens** are prepared by both altering one or more linear **IgE epitopes** and disrupting one or more disulfide bonds of the natural **allergen.** In certain embodiments, the methods of the present invention allow **allergens** to be modified while retaining the ability of the protein to activate T-cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The immunotherapeutics can be prepared in transgenic plants or animals; and administered in injection, aerosol, sublingual or topical form. The immunotherapeutics can also be encoded in gene for gene therapy and delivered by injecting into muscle or skin to induce tolerance. The Examples provided herein use peanut **allergens** to illustrate applications of the invention.

L49 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN

2003:178553 Biochemical, immunological, and structural properties of food **allergens**: What establishes a protein as an **allergen**?. **Bannon, Gary A.** (Product Safety Center, Monsanto, St. Louis, MO, 63167, USA). Abstracts of Papers, 225th ACS National Meeting, New Orleans, LA, United States, March 23-27, 2003, AGFD-039. American Chemical Society: Washington, D. C. (English) 2003. CODEN: 69DSA4.

AB Anal. of a variety of allergenic foods has resulted in the identification of certain biochem. characteristics that are shared by many but not necessarily all food **allergens.** Some of these characteristics include their abundance in the food, their stability to the proteolytic and acidic conditions of the digestive tract, and the ability to promote IgE production and elicit IgE mediated clin. symptoms. The observation that many of the food **allergens** are proteins containing stable tertiary structures that may be important to their allergenicity has led to the assumption that protein structure may be an important factor in the ability of a protein to become an **allergen.** A number of methods have been utilized to gain a better understanding of the structural properties of food **allergens.** These methods include structural anal. by CD spectroscopy, x-ray crystallog., bioinformatics, in vitro digestion assays, and immunol. methods. These methods combined with **IgE epitope** mapping studies have been applied to a number of major food **allergens.** Data from these expts. indicate that there is a link between **allergen** structure and the immunodominant IgE-binding epitopes within a population of food allergic individuals. These results improve our understanding of the clin. relevance of important assays designed to assess the potential allergenicity of novel proteins. Furthermore, the results provide a link between sequence similarity and physicochem. properties of proteins thus enhancing our overall understanding of the characteristics of food **allergens.**

L49 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN
2003:906632 Correction of: 2002:736063 Document No. 139:349665 Correction of: 137:277814 Modified anaphylactic food **allergens** with reduced IgE-binding ability for decreasing clinical reaction to allergy. Caplan, Michael; Sosin, Howard; **Sampson, Hugh**; **Bannon, Gary A.**; **Burks, Wesley A.**; **Cockrell, Gael**; **Compadre, Cesar M.**; Connaughton, Cathie; **Helm, Ricki M.**; King, Nina E.; **Kopper, Randall A.**; **Maleki, Sohelia J.**; Rabjohn, Patrick A.; **Shin, David S.**; Stanley, J. Steven (Panacea Pharmaceuticals, USA; et al.). PCT Int. Appl. WO 2002074250 A2 20020926, 299 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US9108 20020318. PRIORITY: US 2001-PV276822 20010316.

AB It has been determined that **allergens**, which are characterized by both humoral (IgE) and cellular (T-cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by altering as little as a single amino acid within the protein, preferably a hydrophobic residue towards the center of the **IgE epitope**, to eliminate IgE binding. Addnl. or alternatively a modified **allergen** with reduced IgE binding may be prepared by disrupting one or more of the disulfide bonds that are present in the natural **allergen.** The disulfide bonds may be disrupted chemical, e.g., by reduction and alkylation or by mutating one or more cysteine residues present in the primary amino acid sequence of the natural **allergen.** In certain embodiments, modified **allergens** are prepared by both altering one or more linear **IgE epitopes** and disrupting one or more disulfide bonds of the natural **allergen.** In certain embodiments, the methods of the present invention allow **allergens** to be modified while retaining the ability of the protein to activate T-cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The Examples provided herein use peanut **allergens** to illustrate applications of the invention.

L49 ANSWER 5 OF 6 MEDLINE on STN
2002453715. PubMed ID: 12209105. Identification of an 11S globulin as a major hazelnut food **allergen** in hazelnut-induced systemic reactions. Beyer Kirsten; Grishina Galina; Bardina Ludmilla; Grishin Alexander; **Sampson Hugh A.** (Division of Pediatric Allergy and Immunology and the Jaffe Institute for Food Allergy, The Mount Sinai School of Medicine, New York, NY 10029, USA. ) Journal of allergy and clinical immunology, (2002 Sep) 110 (3) 517-23. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Hazelnuts are a common cause of food allergy. Allergic reactions to hazelnuts range from oral allergy syndrome caused by cross-reactivity between tree pollen and hazelnut proteins to severe anaphylactic reactions. Little information is available regarding the identification of pollen-independent hazelnut **allergens.** OBJECTIVE: The aim of the study was to identify these pollen-independent **allergens** in patients with hazelnut allergy with systemic reactions. METHODS: Extracted hazelnut proteins were separated by means of 2-dimensional PAGE, and immunolabeling was performed with individual sera from 14 patients with hazelnut-induced systemic reactions. Edman sequencing was performed on a 40-kd protein identified as an **allergen.** In parallel, RNA isolated from hazelnuts was used to construct a cDNA library. By using the peptide sequence data, oligonucleotide primers were synthesized and used to screen the library. Full-length cDNA clones were isolated, sequenced, expressed, and screened

with patient sera. RESULTS: By using 2-dimensional proteomics, a protein fraction at 40 kd was recognized by serum IgE from 86% (12/14) of the patients with hazelnut allergy with systemic reactions. Two internal amino acid sequences were determined by means of Edman sequencing. Screening of the prepared hazelnut cDNA library with oligonucleotides based on these internal peptide sequences resulted in isolation of a novel protein cDNA. The new protein, named Cor a 9, belongs to the 11S globulin seed storage protein family. This family comprises known food **allergens** in peanut (Ara h 3) and soybean (glycine max). The pairwise homology among these 3 proteins ranges from 45% to 50%. Interestingly, one known IgE-binding epitope of Ara h 3 shares 67% of homologous amino acid residues with the corresponding area of Cor a 9. The amino acids that differ were previously shown not to be critical for IgE binding in Ara h 3. CONCLUSION: Cor a 9 is the first tree pollen-unrelated hazelnut **allergen** isolated, sequenced, and cloned. The identification of food **allergens** is the first step toward generating recombinant **allergens** for use in future immunotherapeutic approaches. In addition, the detection of conserved **IgE epitopes** in common food **allergens**, such as seed storage proteins, might be a useful tool for predicting cross-reactivity to certain foods.

L49 ANSWER 6 OF 6 MEDLINE on STN

2001486998. PubMed ID: 11529896. Identification of IgE and IgG binding epitopes on beta- and kappa-casein in cow's milk allergic patients. Chatchatee P; Jarvinen K M; Bardina L; Vila L; Beyer K; **Sampson H A.** (Division of Paediatric Allergy & Immunology, Jaffe Institute for Food Allergy, The Mount Sinai Medical Center, New York, NY, USA. ) Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (2001 Aug) 31 (8) 1256-62. Journal code: 8906443. ISSN: 0954-7894. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: Cow's milk allergy (CMA) affects 2.5% of children aged less than 2 years of age. Although beta- and kappa-casein are considered among the major **allergens** responsible for CMA, no data are available on their allergenic epitopes in humans. OBJECTIVE: The aim of the study was to identify IgE- and IgG-binding epitopes on beta- and kappa-casein and to determine whether the pattern of epitope recognition is associated with the natural history of CMA. METHODS: Overlapping decapeptides representing the entire length of beta- and kappa-casein, respectively, were synthesized on a cellulose-derivatized membrane. Sera from 15 milk-allergic children, 4-18 years of age, with high levels of specific IgE antibodies to cow's milk were used to identify IgE- and IgG-binding epitopes. In addition, **IgE epitopes** were screened with pooled or individual sera from younger patients aged less than 3 years and who had low levels of specific serum IgE, who are likely to outgrow CMA. RESULTS: Six major and three minor IgE-binding epitopes, as well as eight major and one minor IgG binding regions, were identified on beta-casein. Eight major IgE-binding epitopes, as well as two major and two minor IgG-binding epitopes, were detected on kappa-casein. Three of the IgE binding regions on beta-casein and six on kappa-casein were recognized by the majority of patients in the older age group, but not by the younger patients. CONCLUSION: Information regarding the immunodominant epitopes in beta- and kappa-casein may be important for understanding the pathophysiology and natural history of CMA. Differences in epitope recognition may be useful in identifying children who will have persistent milk hypersensitivity.

=>

---Logging off of STN---

=>
Executing the logoff script...

=> LOG Y

| COST IN U.S. DOLLARS | SINCE FILE ENTRY | TOTAL SESSION |
|---|---|---|
| FULL ESTIMATED COST | 418.35 | 418.56 |

| DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS) | SINCE FILE ENTRY | TOTAL SESSION |
|---|---|---|
| CA SUBSCRIBER PRICE | -18.02 | -18.02 |

STN INTERNATIONAL LOGOFF AT 08:59:56 ON 25 MAY 2004